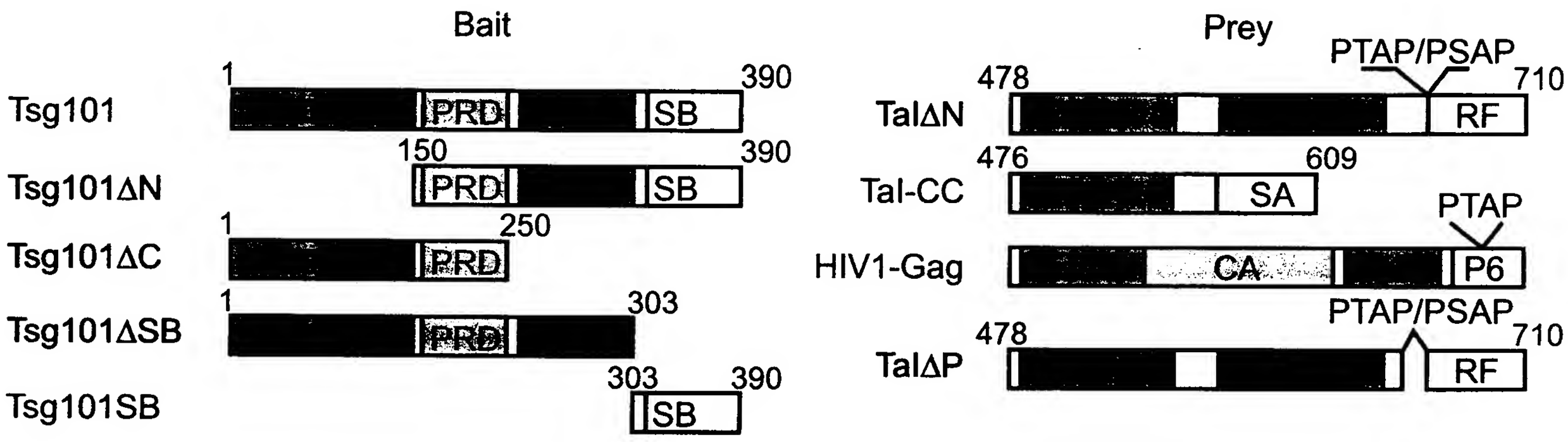

Bait
Prey
Tsg101
1
390
PRD
SB
Tsg101ΔN
150
390
Tsg101ΔC
250
Tsg101ΔSB
303
Tsg101SB
303
390
TalΔN
478
PTAP/PSAP
710
RF
Tal-CC
476
609
SA
HIV1-Gag
PTAP
CA
P6
TalΔP

Fig. 1a

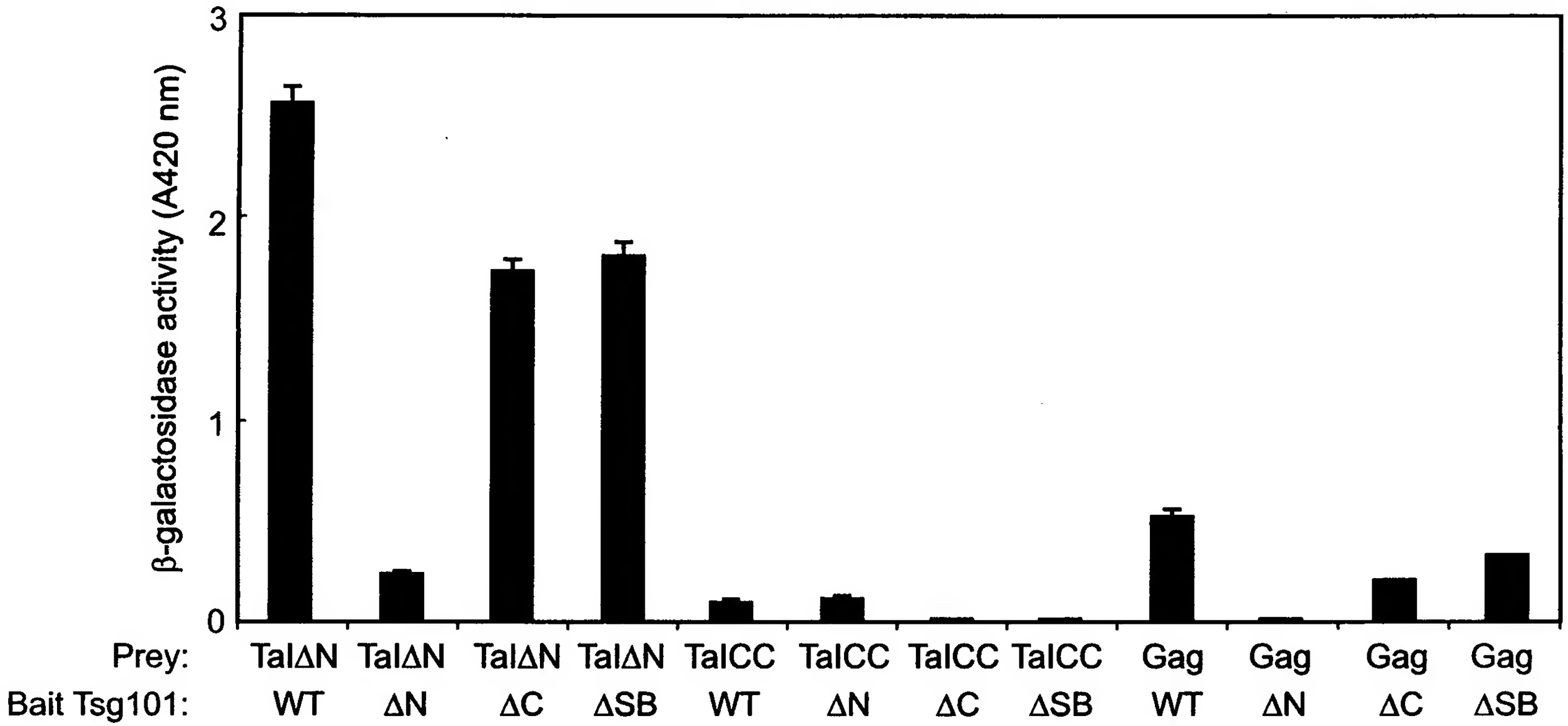

β-galactosidase activity (A420 nm)
3
2
1
0
Prey:
Bait Tsg101:
TalΔN WT
TalΔN ΔN
TalΔN ΔC
TalΔN ΔSB
TalCC WT
TalCC ΔN
TalCC ΔC
TalCC ΔSB
Gag WT
Gag ΔN
Gag ΔC
Gag ΔSB

Fig. 1b

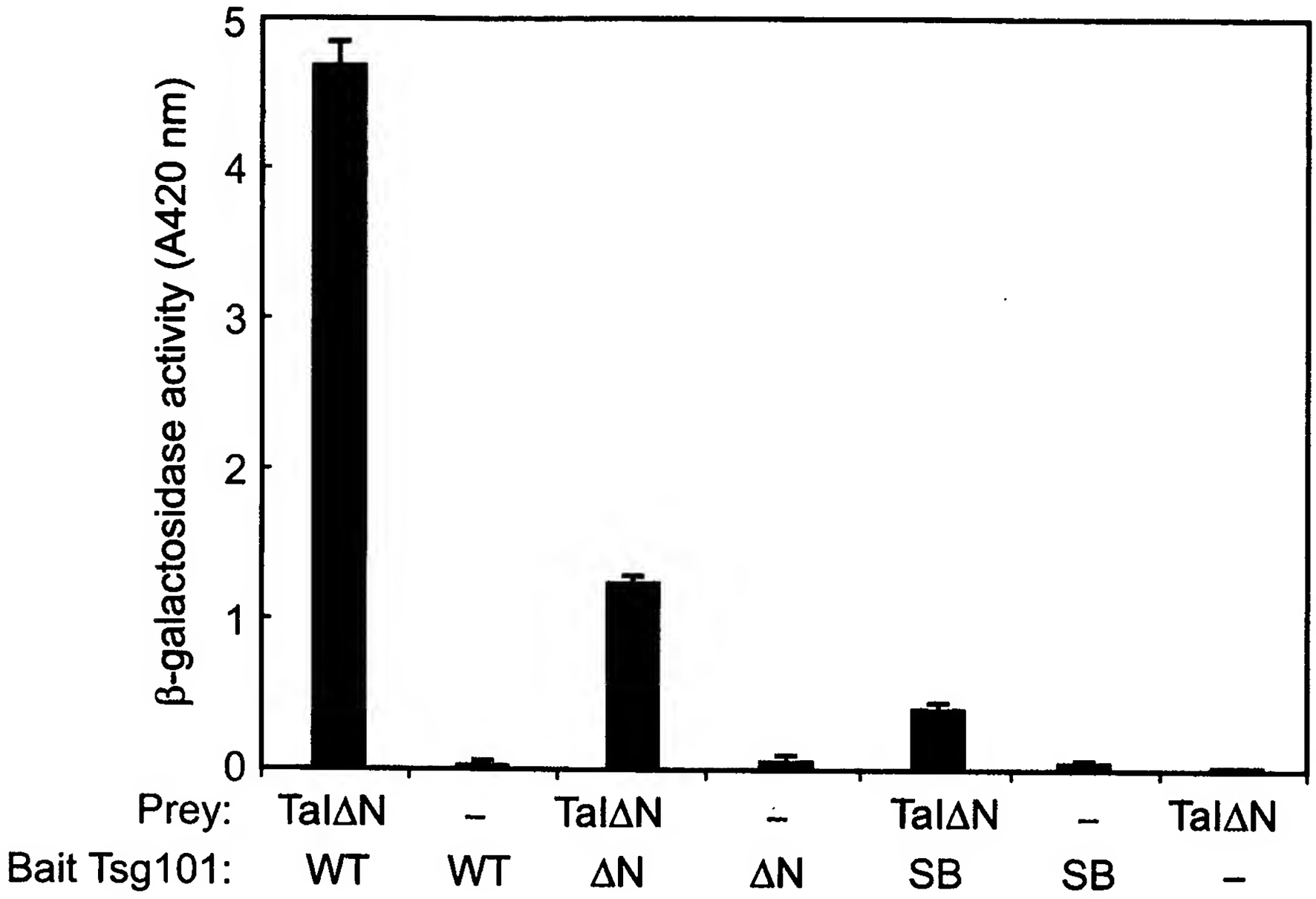

β-galactosidase activity (A420 nm)
5
4
3
2
1
0
Prey: TalΔN – TalΔN – TalΔN – TalΔN
Bait Tsg101: WT WT ΔN ΔN SB SB –

Fig. 1c

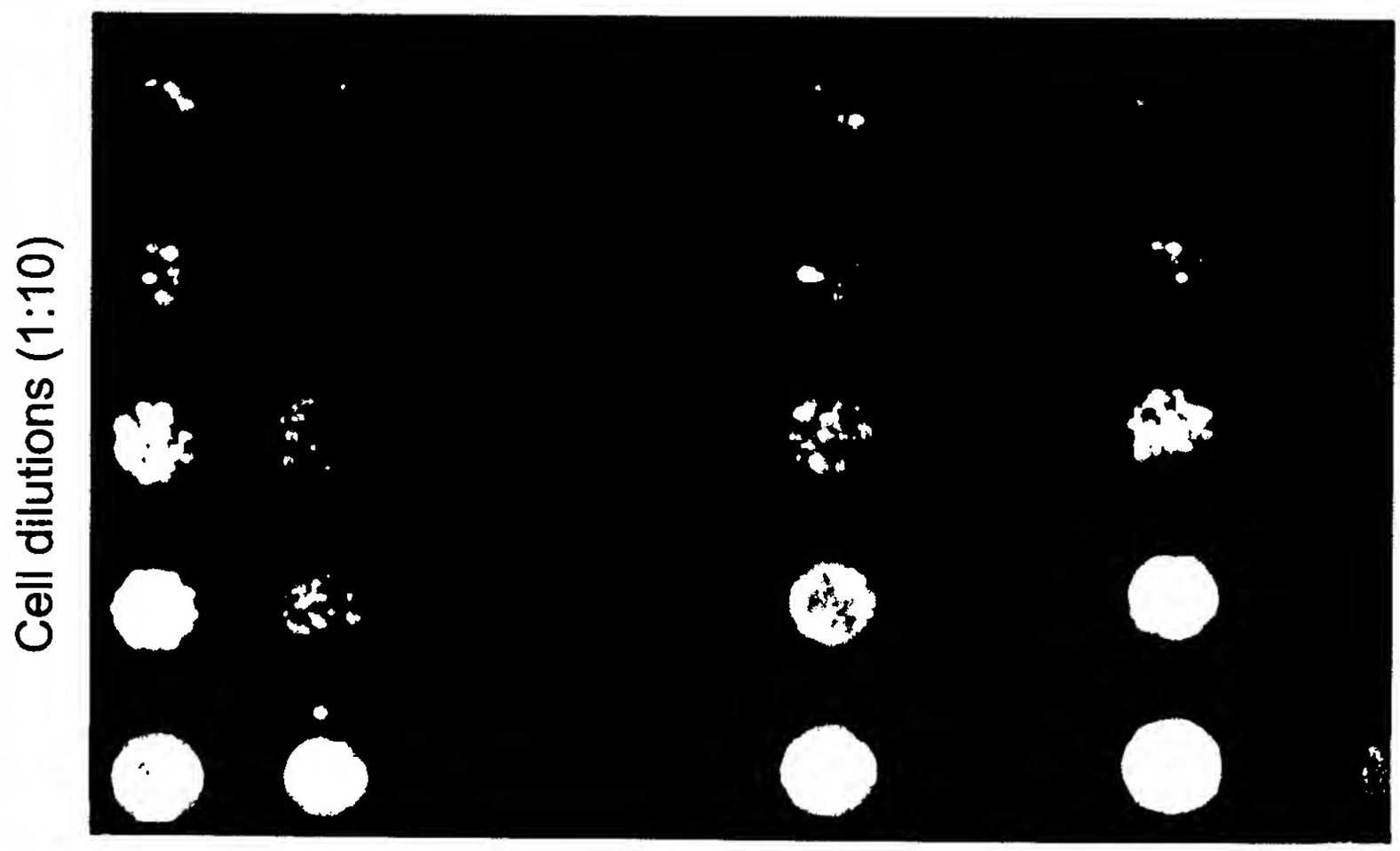

Cell dilutions (1:10)
Prey: ΔN ΔP ΔN ΔP ΔN ΔP ΔN ΔP
Bait Tsg101: WT WT ΔN ΔN ΔSB ΔSB ΔC ΔC

Fig. 1d

```
   1  MPLFFRKRKP SEEARKRLEY QMCLAKEAGA DDILDISKCE LSEIPFGAFA
  51  TCKVLQKKVL IVHTNHLTSL LPKSCSLLSL ATIKVLDLHD NQLTALPDDL
 101  GQLTALQVLN VERNQLMQLP RSIGNLTQLQ TLNVKDNKLK ELPDTVGELR
 151  SLRTLNISGN EIQRLPQMLA HVRTLEMLSL DASAMVYPPR EVCGAGTAAI
 201  LQFLCKESGL EYYPPSQYLL PILEQDGIEN SRDSPDGPTD RFSREELEWQ
 251  NRFSDYEKRK EQKMLEKLEF ERRLELGQRE HTQLLQQSSS QKDEILQTVK
 301  EEQSRLEQGL SEHQRHLDAE RQRLQEQLKQ TEQNISSRIQ KLLQDNQRQK
 351  KSSEILKSLE NERIRMEQLM SITQEETESL RRRDVASAMQ QMLTESCKNR
 401  LIQMAYESQR QNLVQQACSS MAEMDERFQQ ILSWQQMDQN KAISQILQES
 451  AMQKAAFEAL QVKKDLMHRQ IRSQIKLIET ELLQLTQLEL KRKSLDTESL
 501  QEMISEQRWA LSSLLQQLLK EKQQREEELR EILTELEAKS ETRQENYWLI
 551  QYQRLLNQKP LSLKLQEEGM ERQLVALLEE LSAEHYLPIF AHHRLSLDLL
 601  SQMSPGDLAK VGVSEAGLQH EILRRVQELL DAARIQPELK PPMGEVVT[redacted]
 651  [redacted]QEPPESVR [redacted]PAELEV QASECVVCLE REAQMIFLNC GHVCCCQQCC
 701  QPLRTCPLCR QDIAQRLRIY HSS
```

Fig. 2a

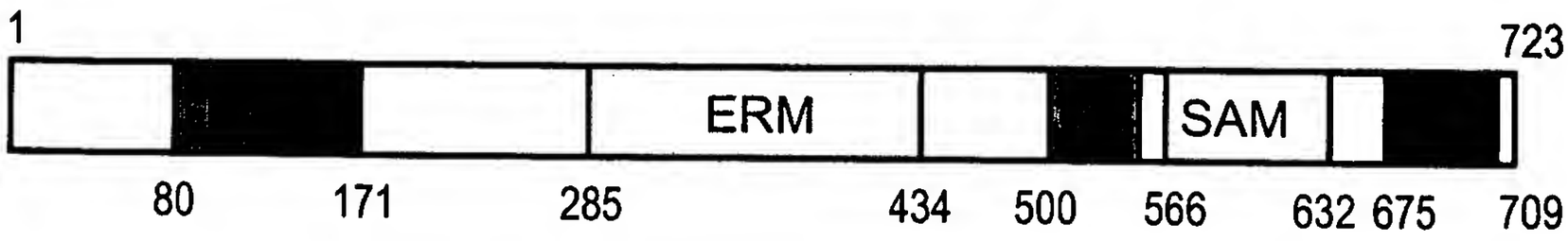


Fig. 2b

Fig. 2c

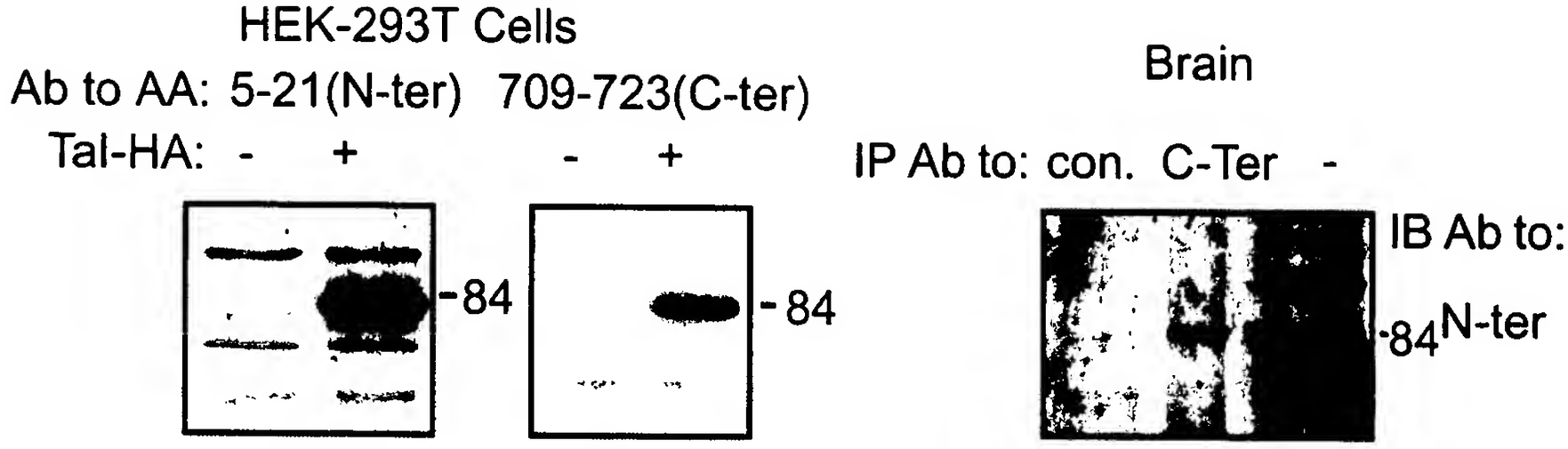


Fig. 2d

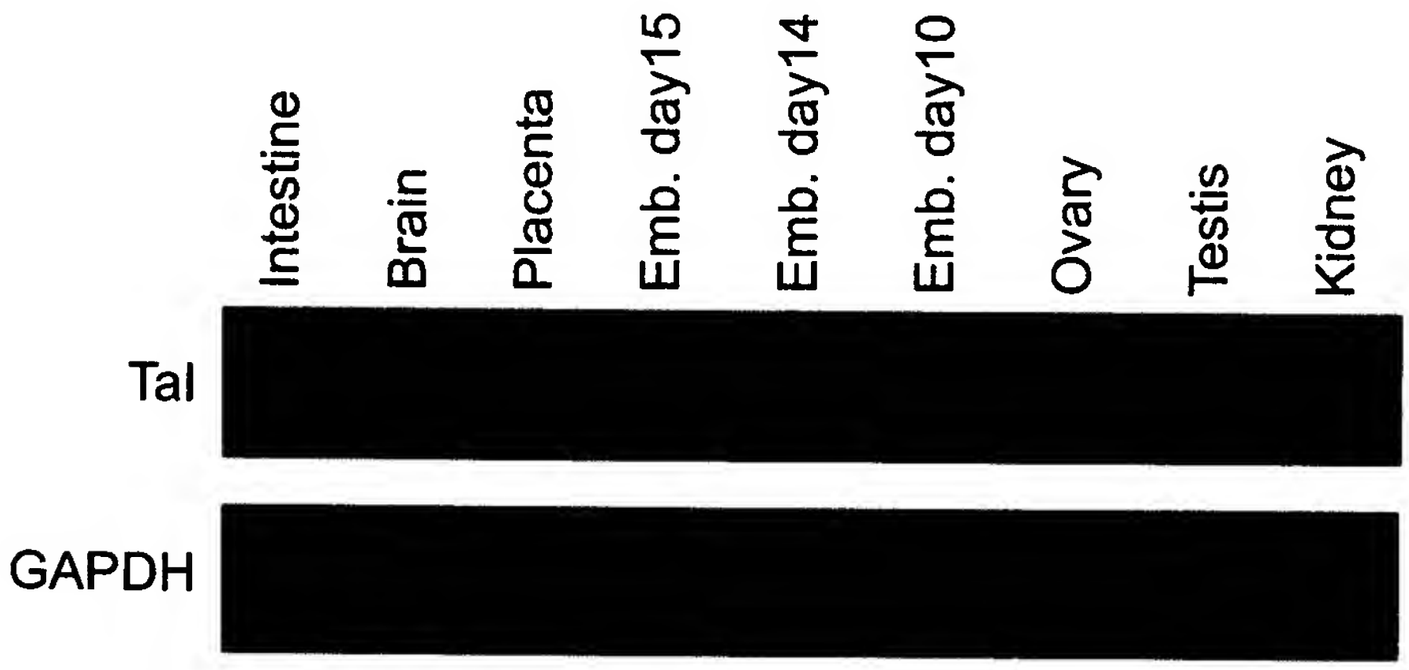


Fig. 2e

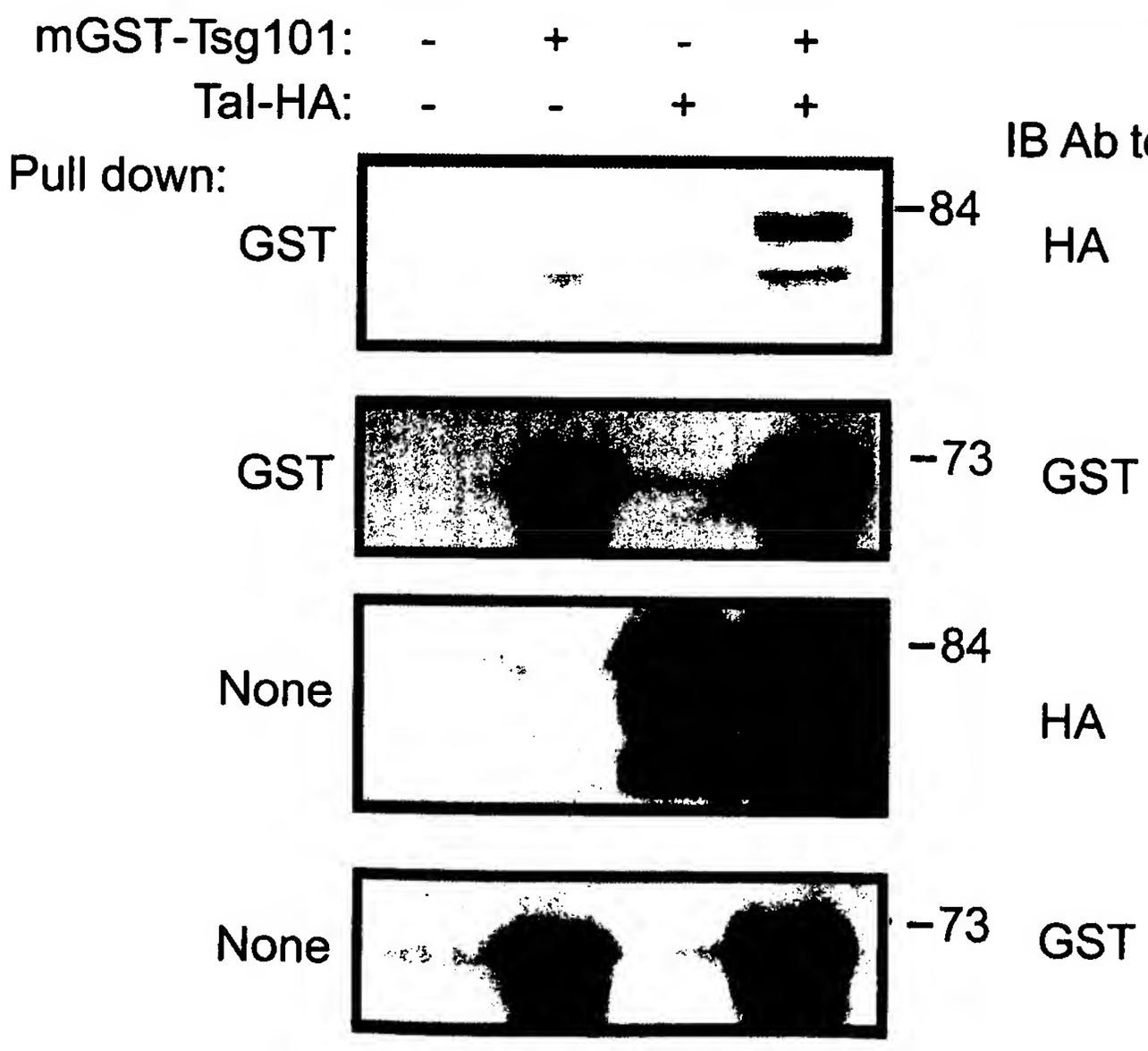


Fig. 3a

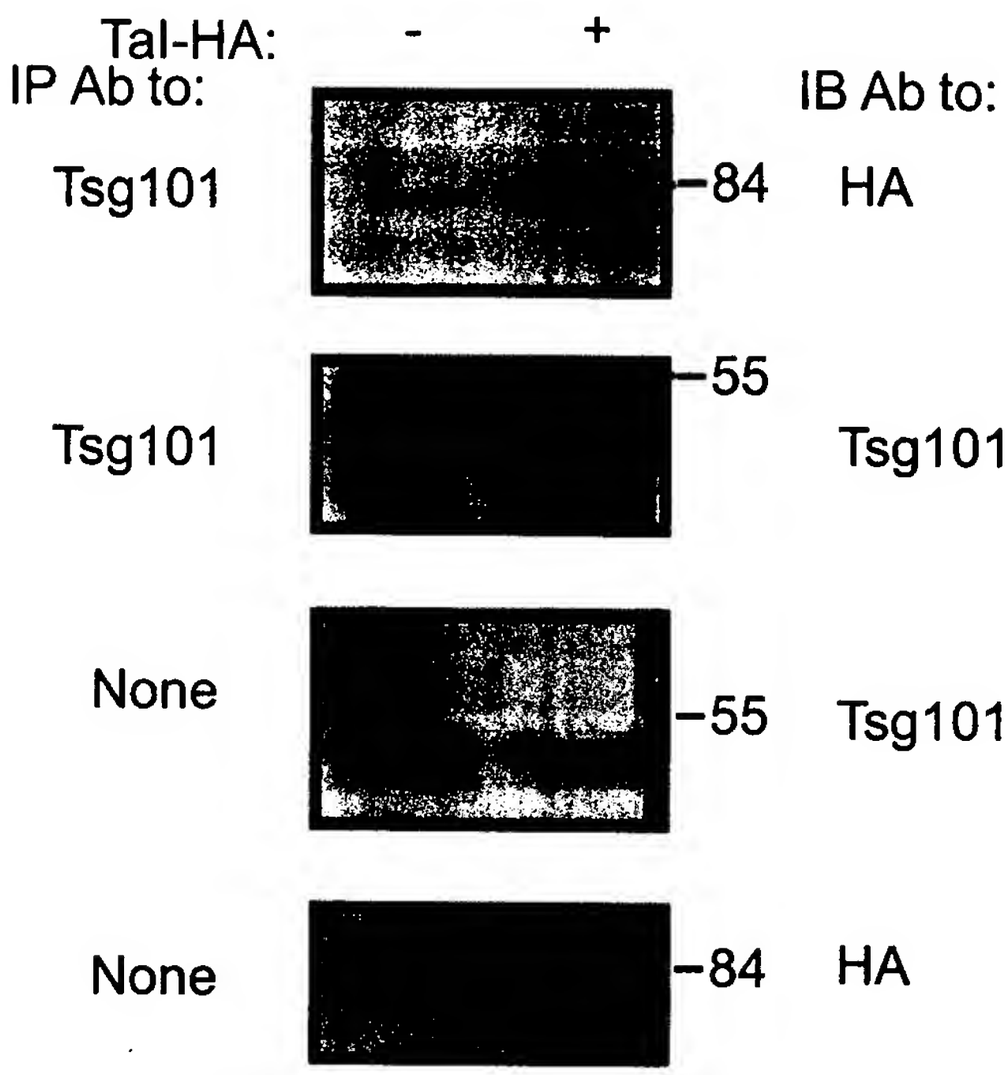

Tal-HA: - +
IP Ab to:
IB Ab to:
Tsg101
84
HA
Tsg101
55
Tsg101
None
55
Tsg101
None
84
HA

Fig. 3b

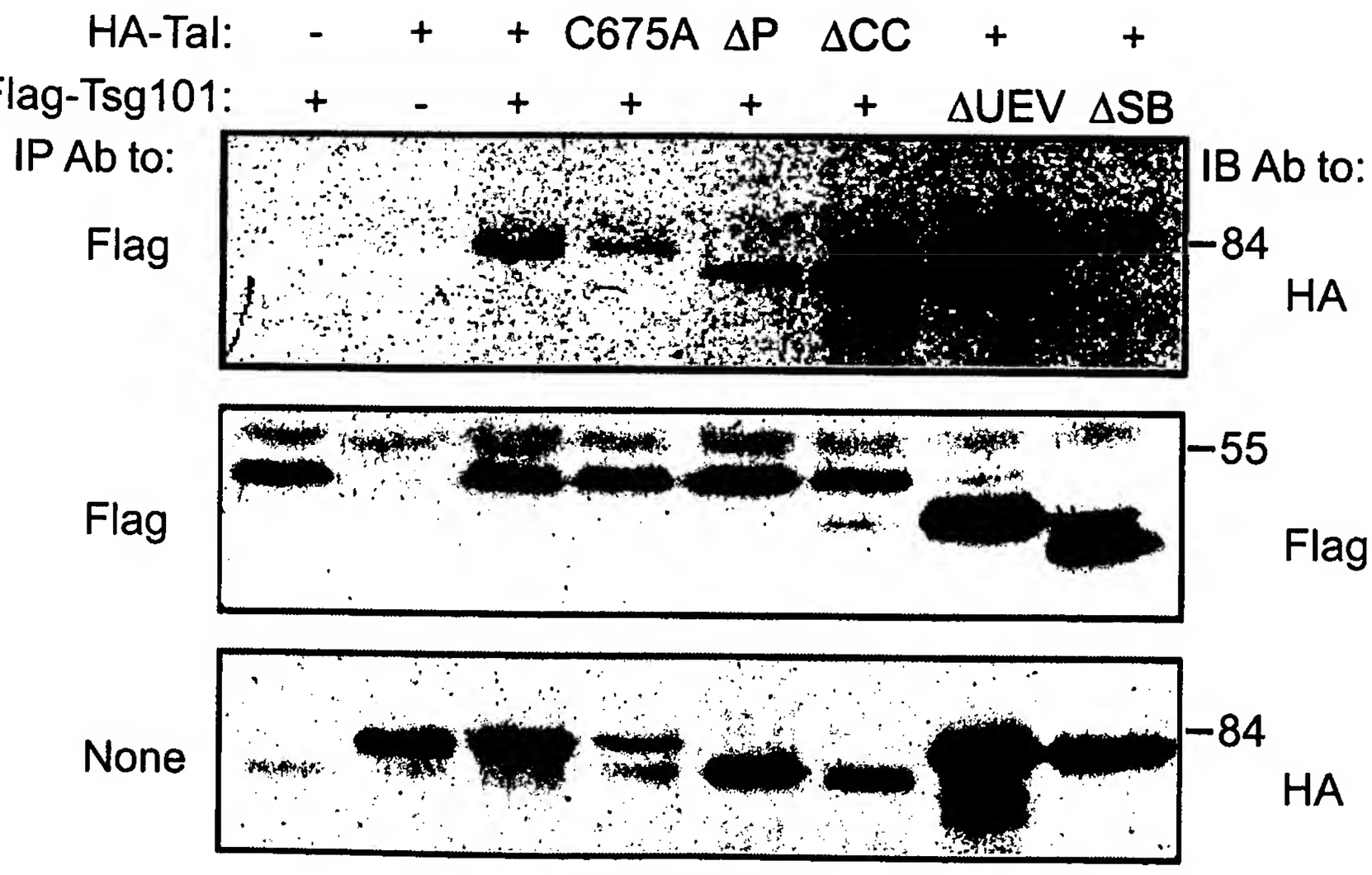

HA-Tal: - + + C675A ΔP ΔCC + +
Flag-Tsg101: + - + + + + ΔUEV ΔSB
IP Ab to:
IB Ab to:
Flag
84
HA
Flag
55
Flag
None
84
HA

Fig. 3c

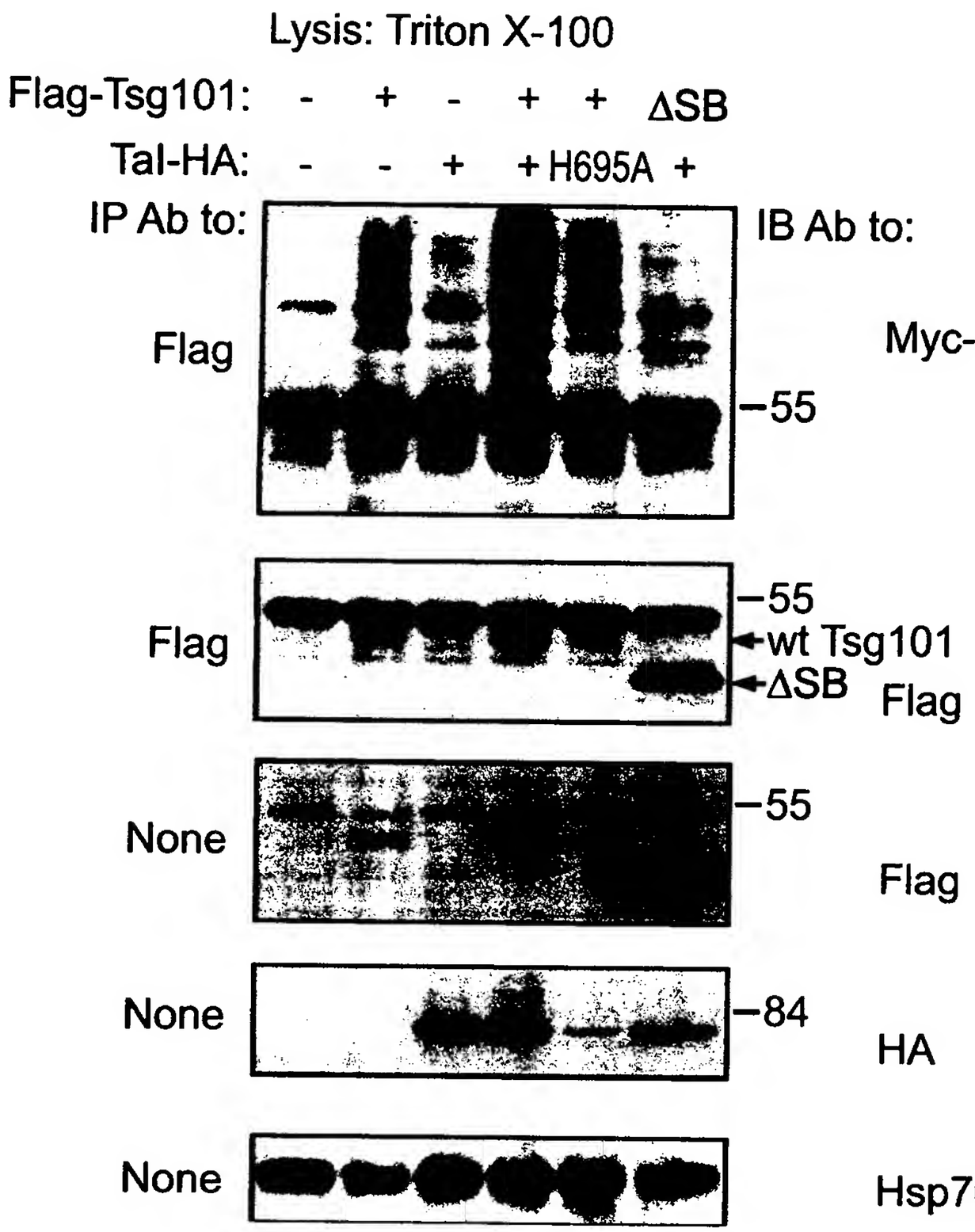

Lysis: Triton X-100
Flag-Tsg101: - + - + + ΔSB
Tal-HA: - - + + H695A +
IP Ab to:
IB Ab to:
Flag
Myc-Ub
55
Flag
55
wt Tsg101
ΔSB
Flag
None
55
Flag
None
84
HA
None
Hsp70

Fig. 4a

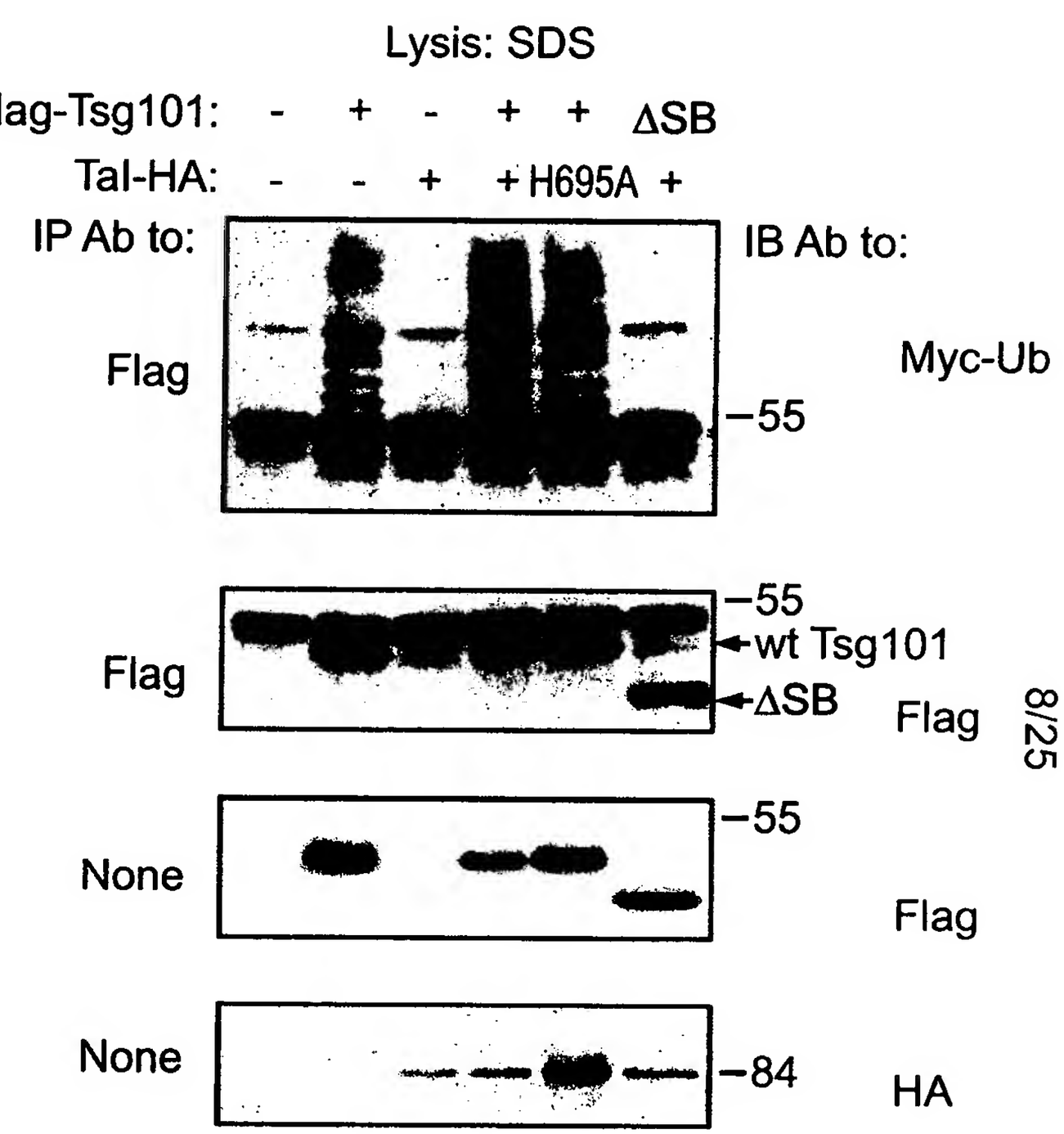

Lysis: SDS
Flag-Tsg101: - + - + + ΔSB
Tal-HA: - - + + H695A +
IP Ab to:
IB Ab to:
Flag
Myc-Ub
55
Flag
55
wt Tsg101
ΔSB
Flag
None
55
Flag
None
84
HA

Fig. 4b

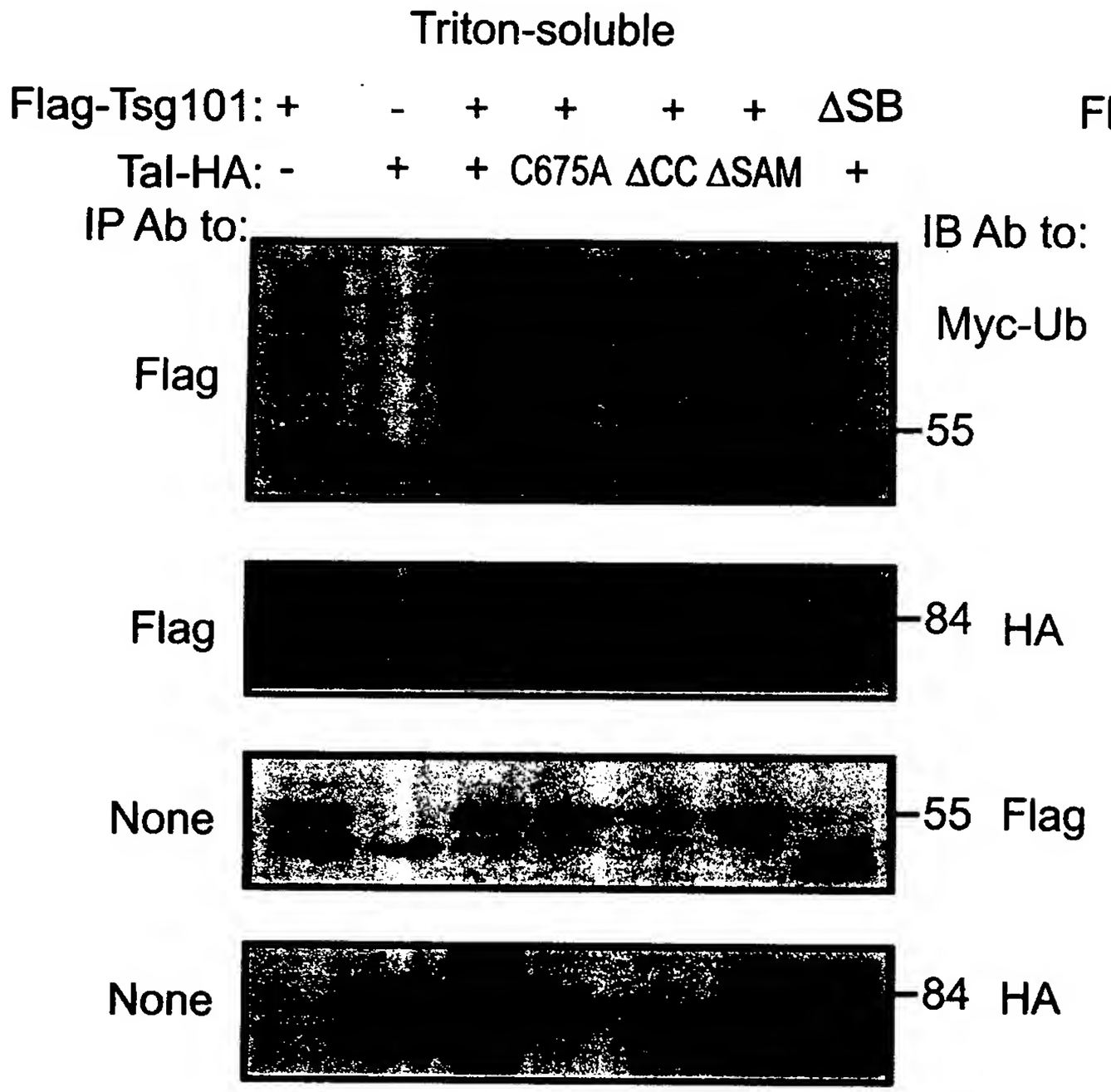

Triton-soluble
Flag-Tsg101: + - + + + + ΔSB
Tal-HA: - + + C675A ΔCC ΔSAM +
IP Ab to:
IB Ab to:
Flag
Myc-Ub
55
Flag
84
HA
None
55
Flag
None
84
HA

Fig. 4c

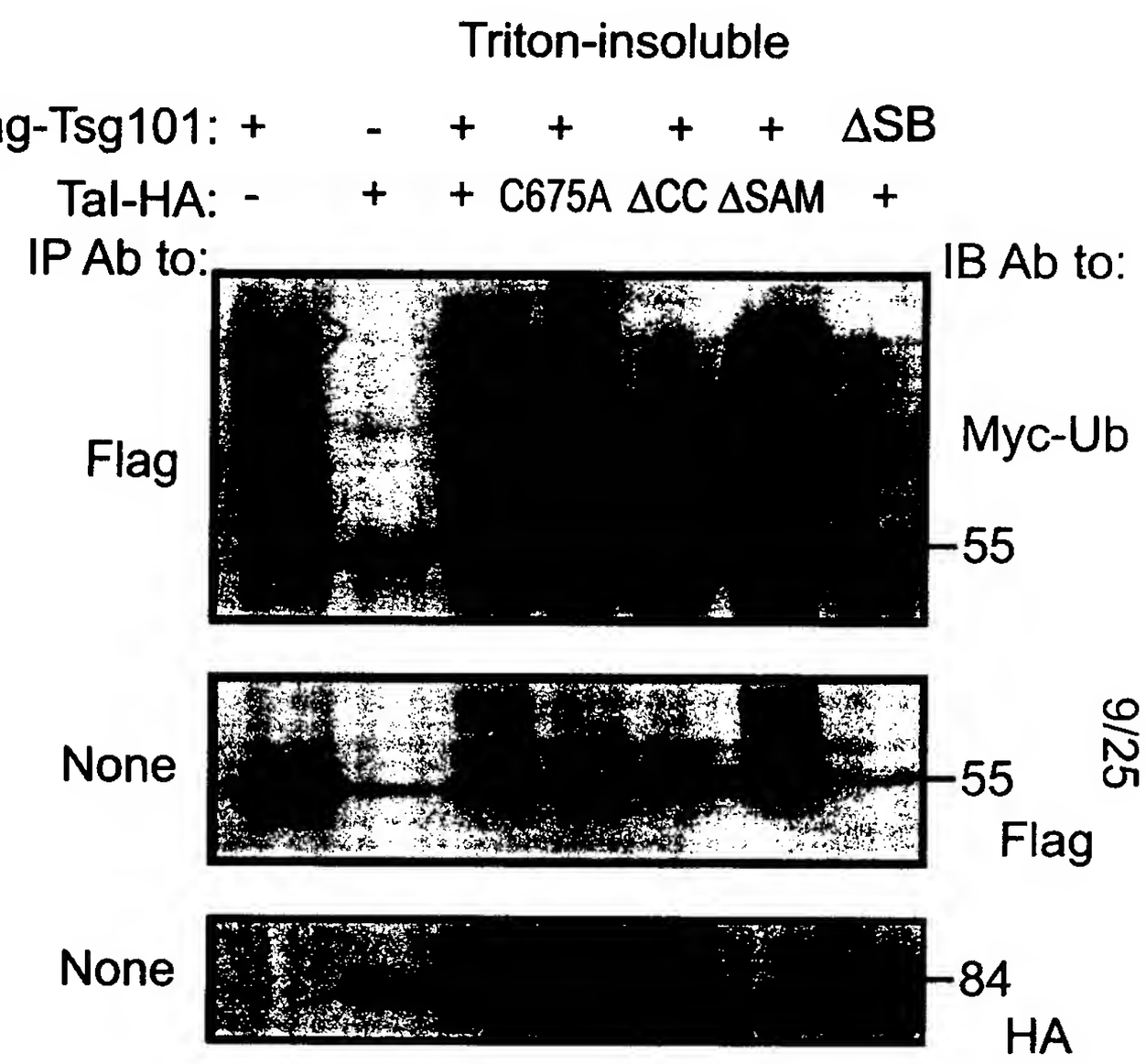

Triton-insoluble
Flag-Tsg101: + - + + + + ΔSB
Tal-HA: - + + C675A ΔCC ΔSAM +
IP Ab to:
IB Ab to:
Flag
Myc-Ub
55
None
55
Flag
None
84
HA

Fig. 4d

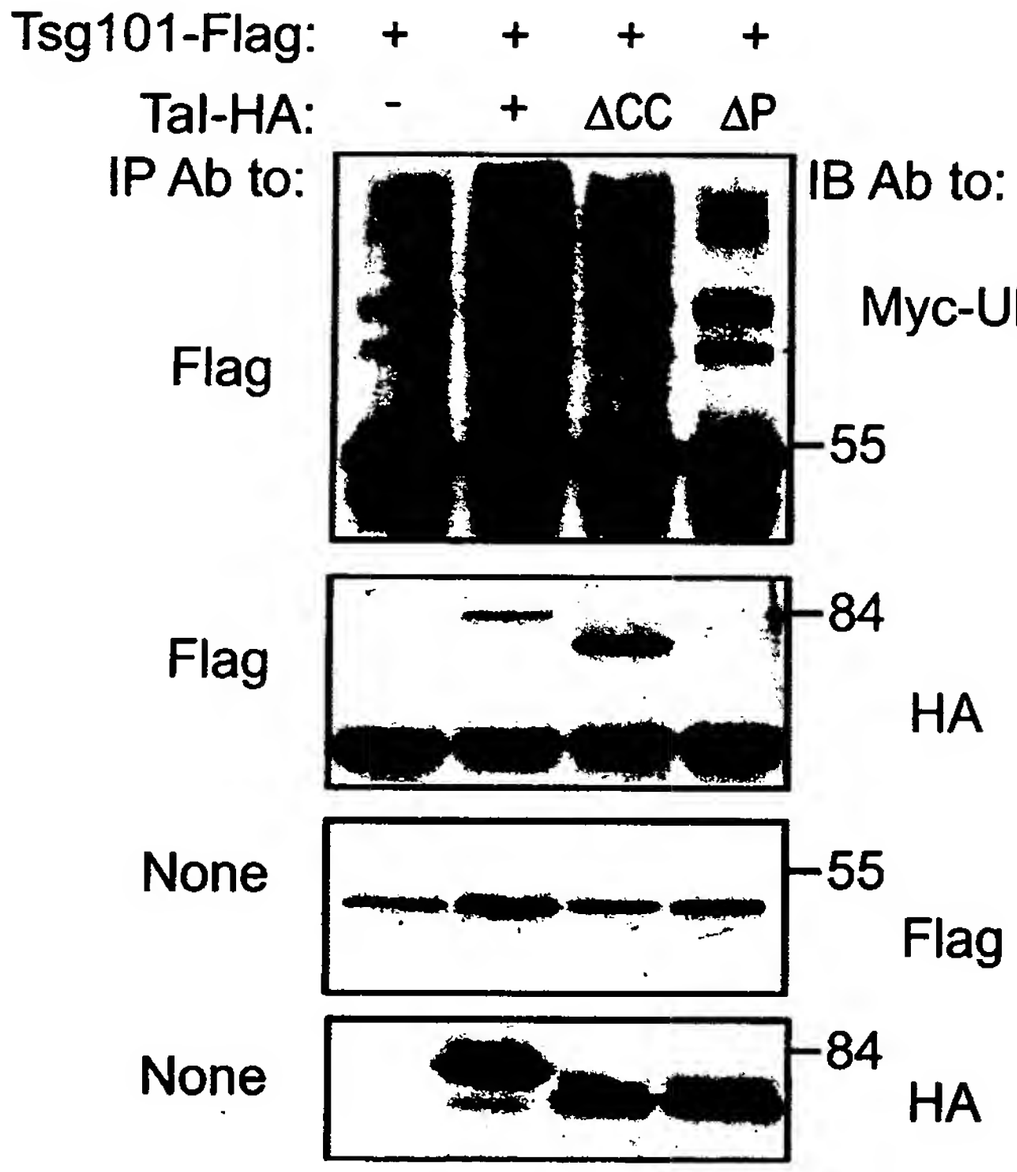


Fig. 5a

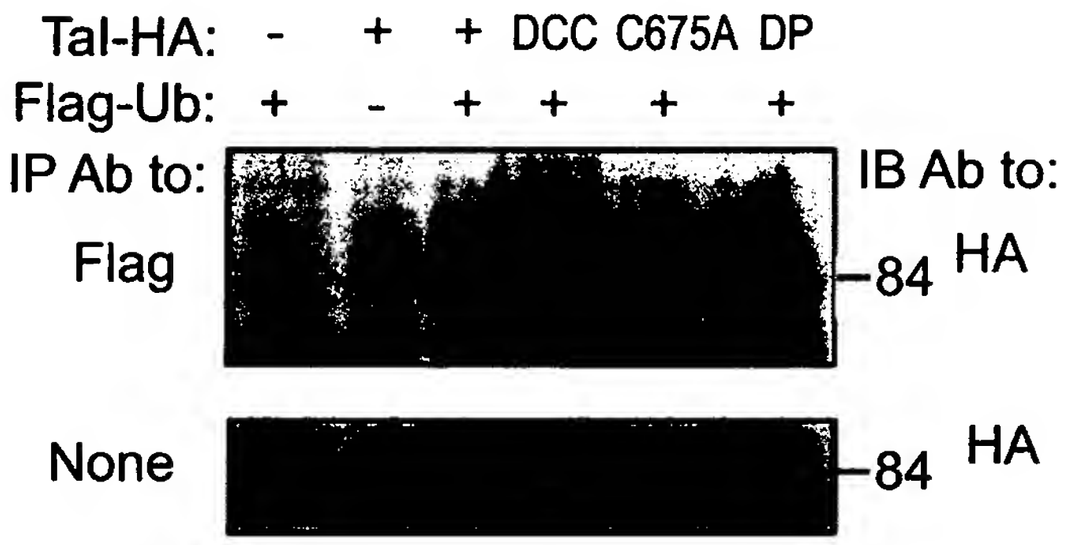


Fig. 5b

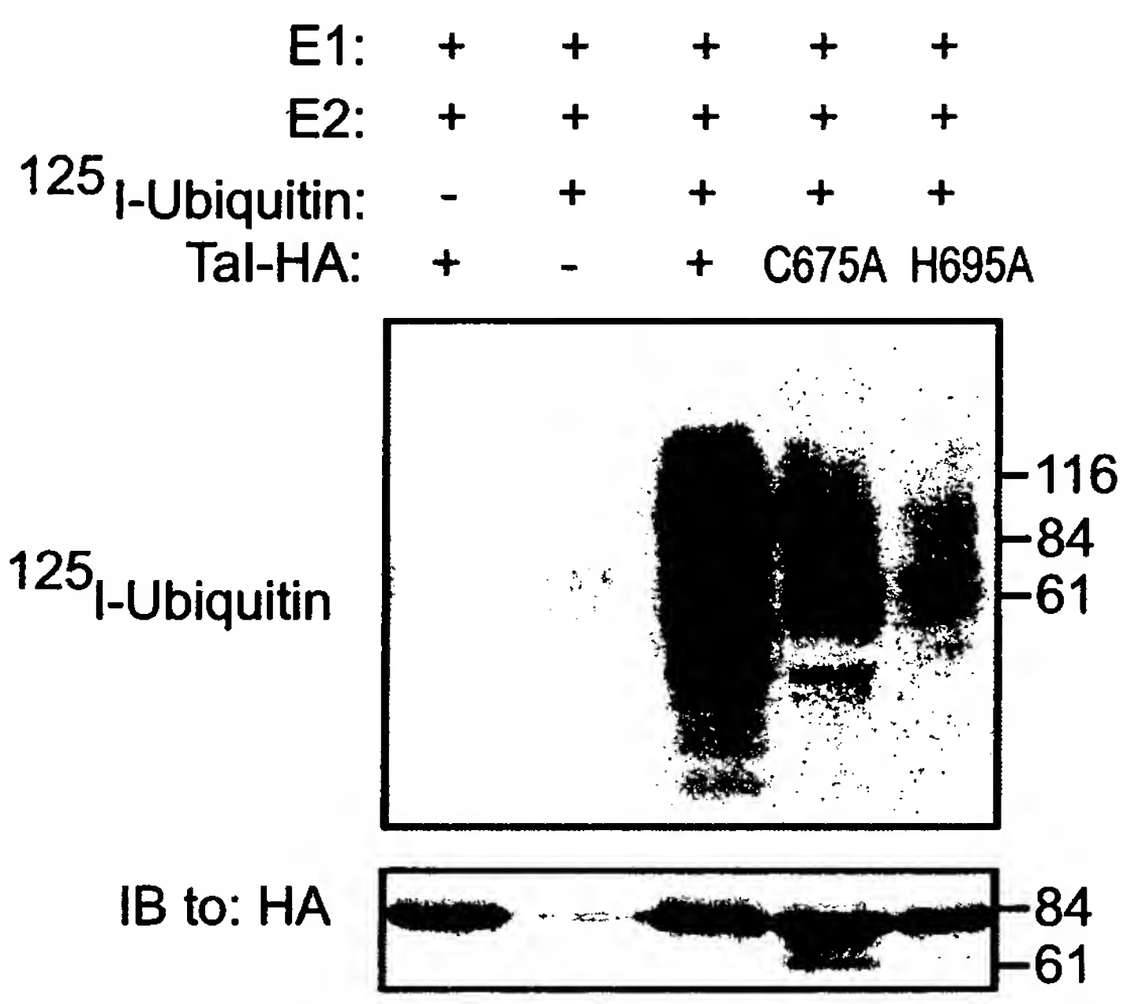


Fig. 5c

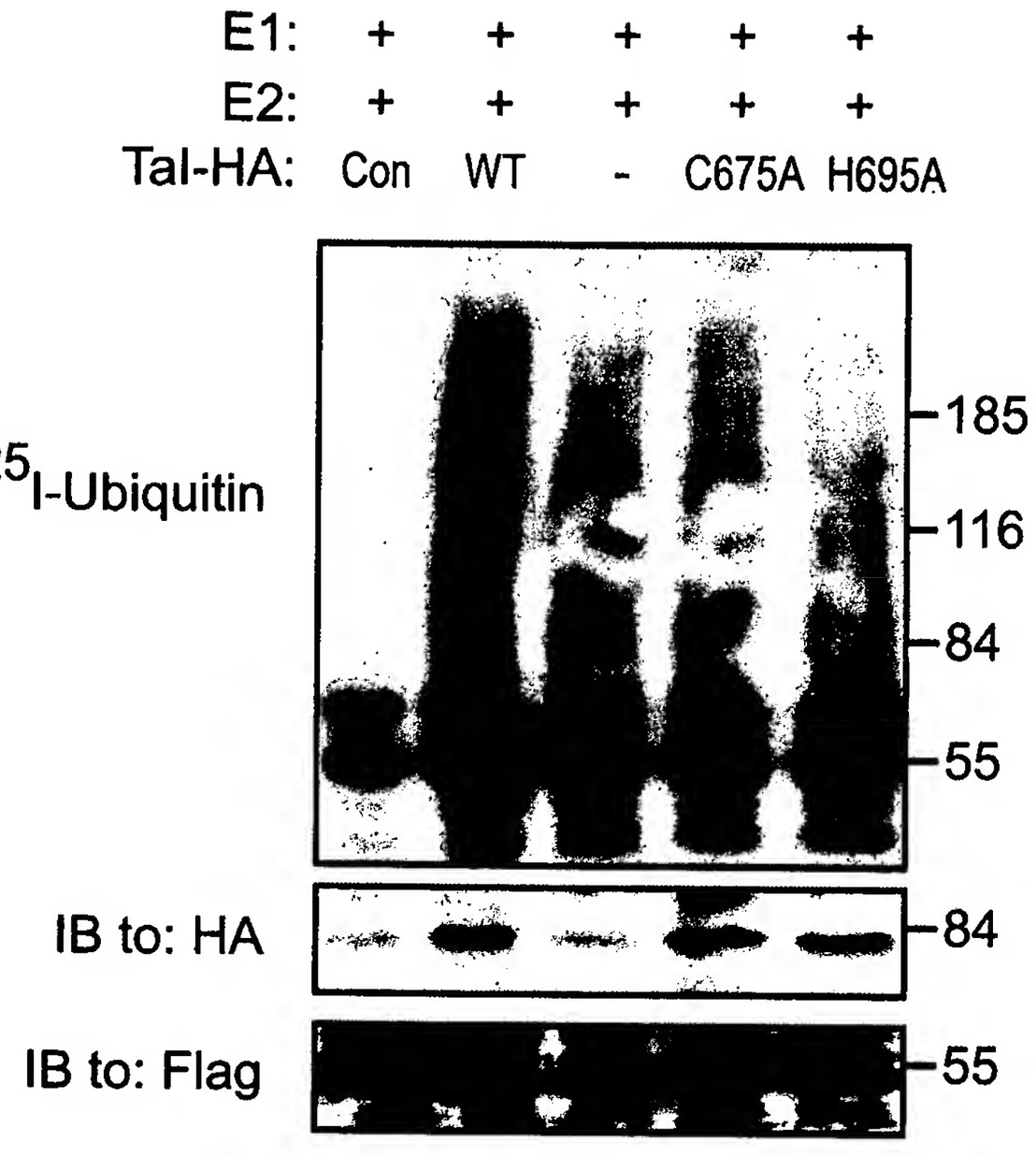

E1: + + + + +
E2: + + + + +
Tal-HA: Con WT - C675A H695A
125I-Ubiquitin
185
116
84
55
IB to: HA
84
IB to: Flag
55

Fig. 5d

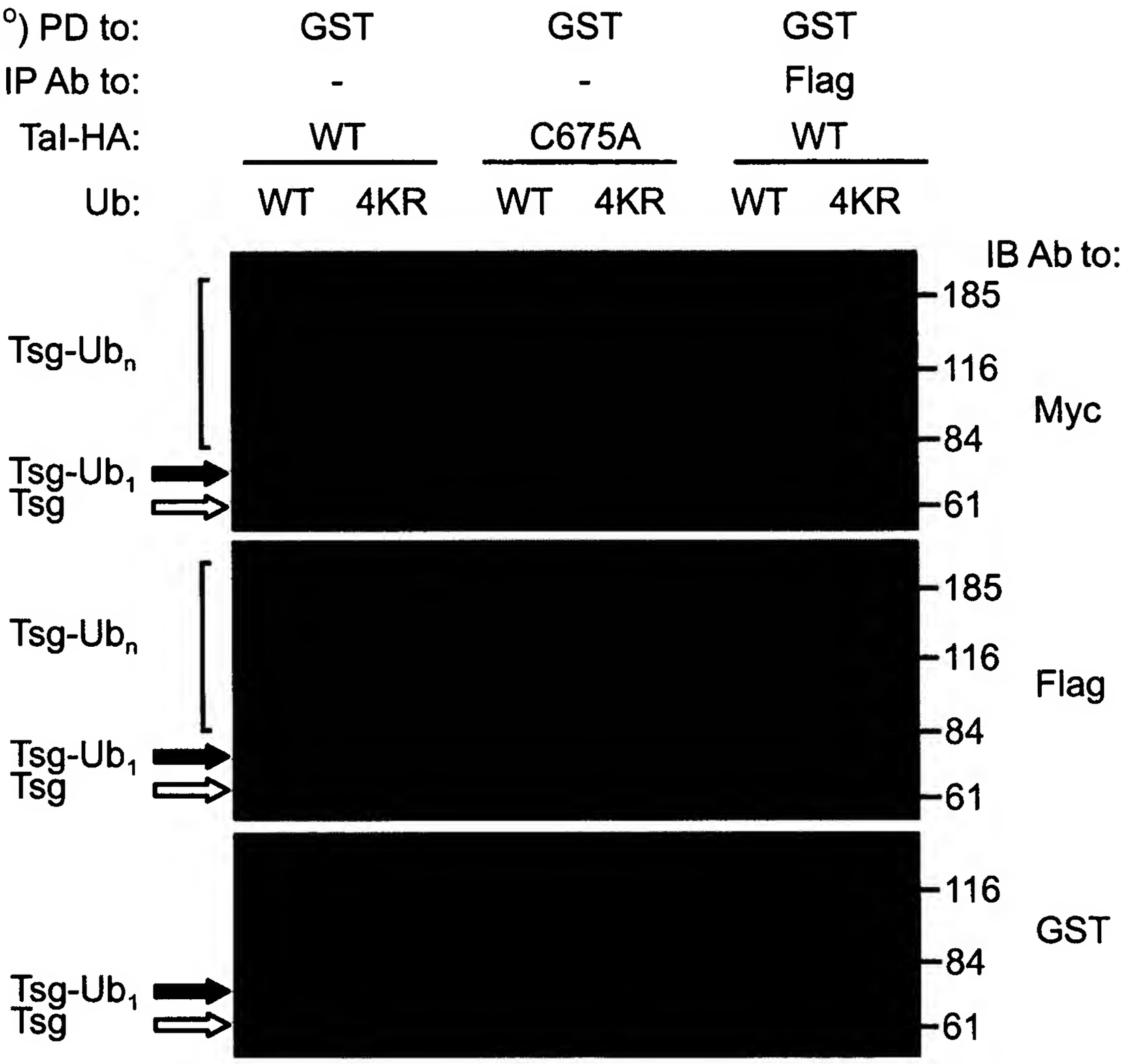

(1°) PD to:
GST
GST
GST
(2°) IP Ab to:
-
-
Flag
Tal-HA:
WT
C675A
WT
Ub:
WT
4KR
WT
4KR
WT
4KR
IB Ab to:
Tsg-Ub$_n$
Tsg-Ub$_1$
Tsg
185
116
84
61
Myc
Tsg-Ub$_n$
Tsg-Ub$_1$
Tsg
185
116
84
61
Flag
116
84
61
GST
Tsg-Ub$_1$
Tsg

Fig. 5e

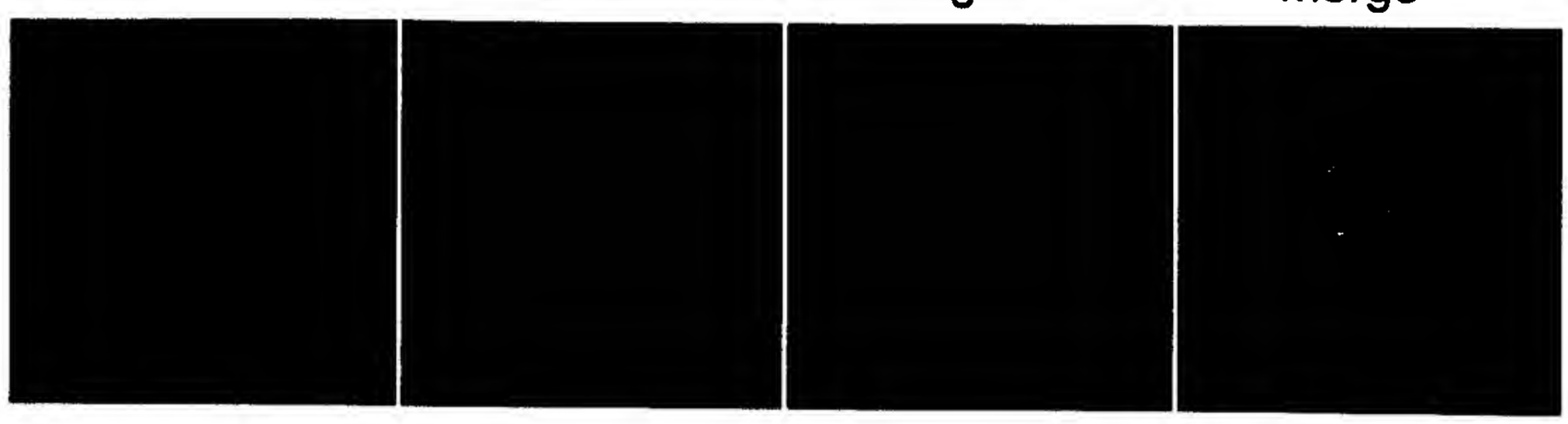


Fig. 6a

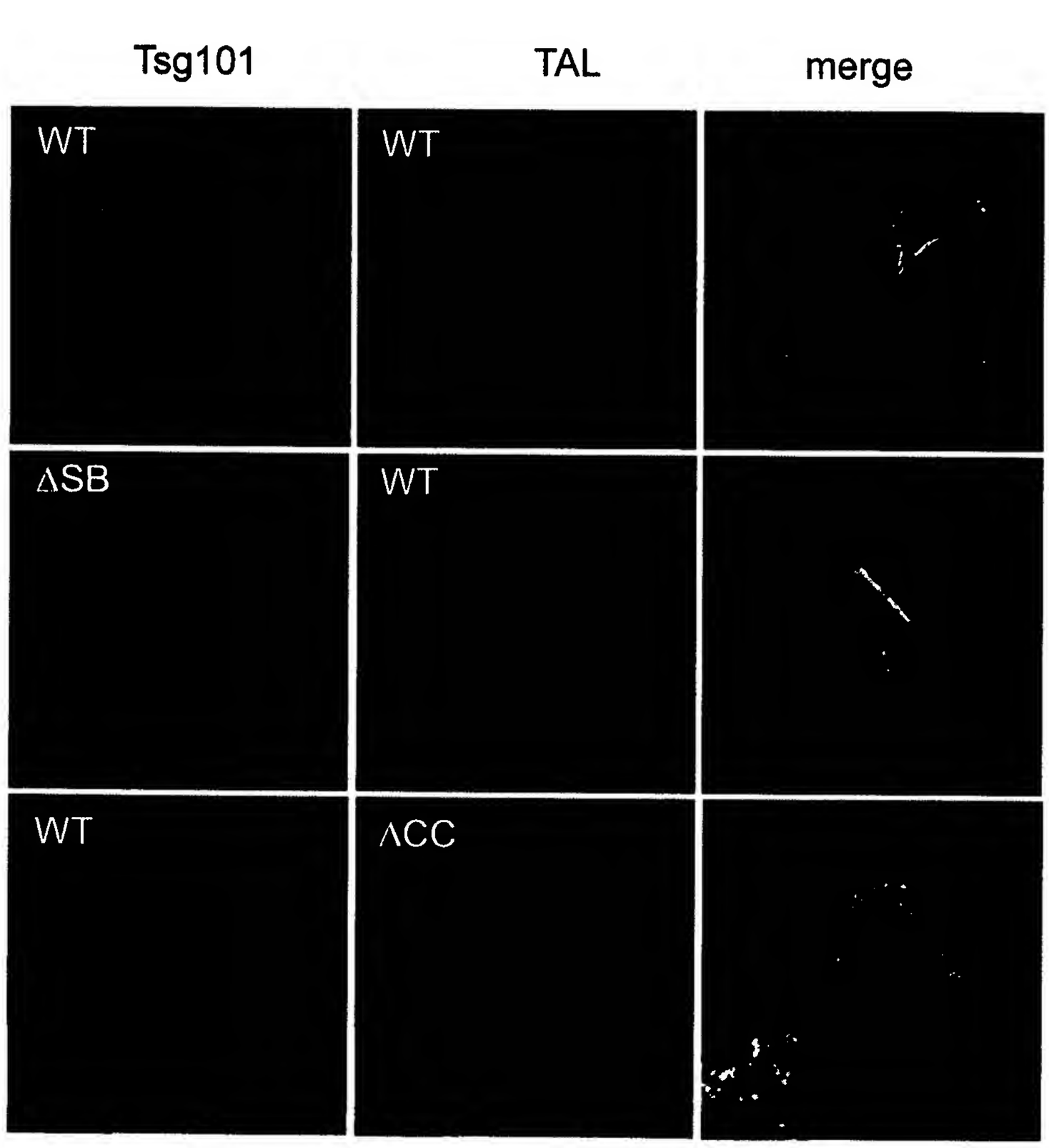


Fig. 6b

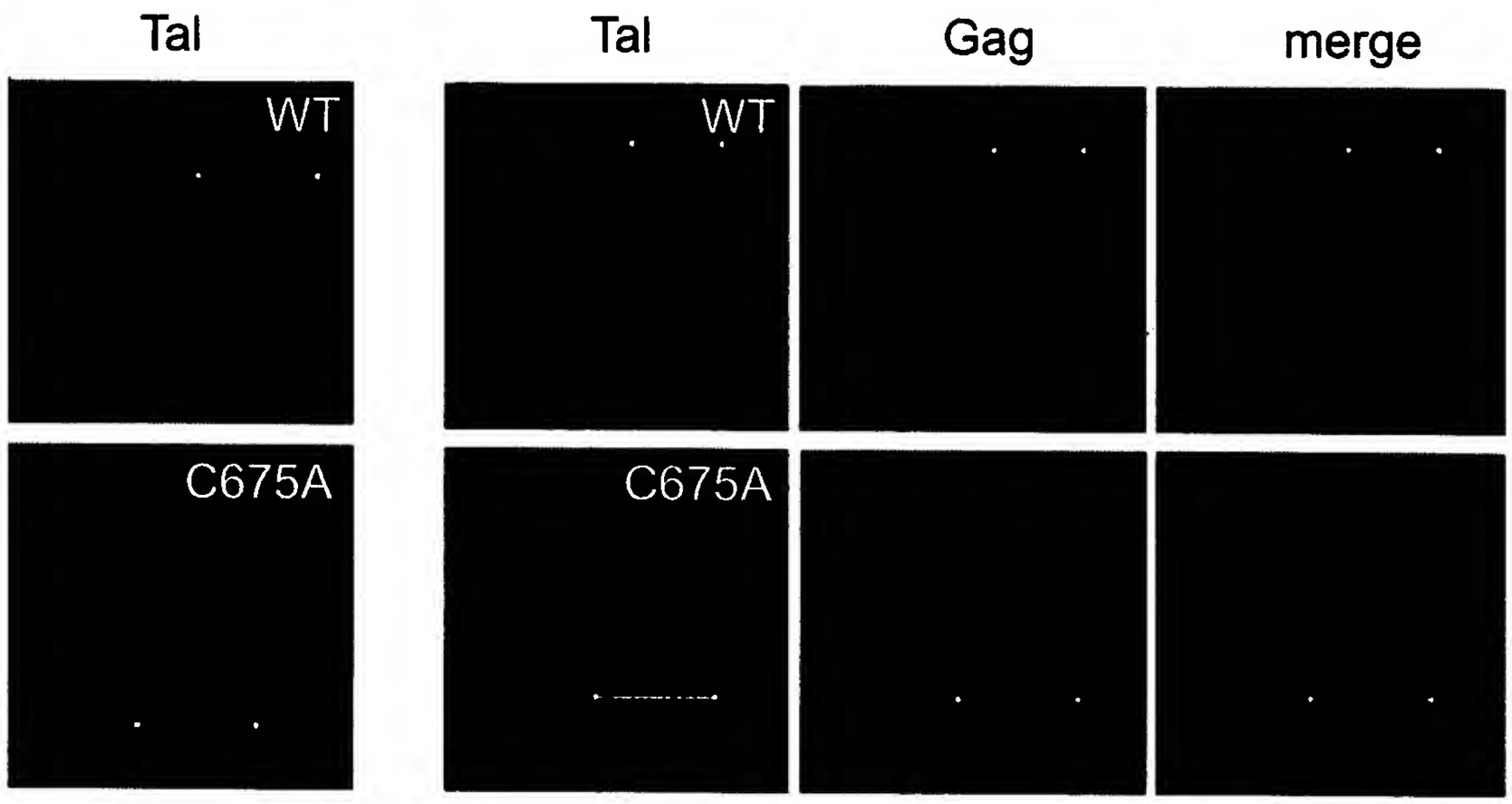


Fig. 6c

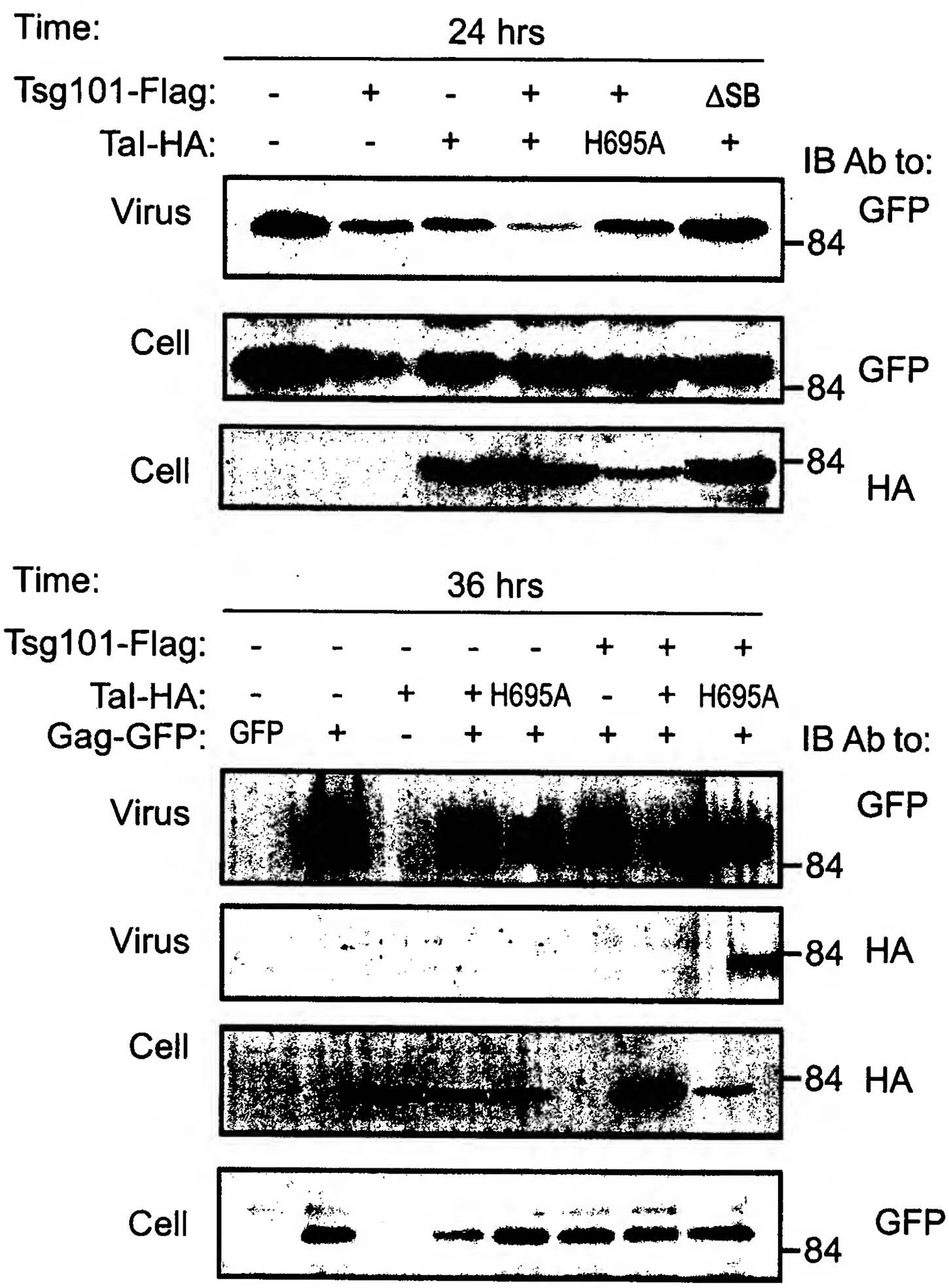

Time:
24 hrs
Tsg101-Flag: - + - + + ΔSB
Tal-HA: - - + + H695A +
IB Ab to:
Virus
GFP
84
Cell
GFP
84
Cell
84
HA
Time:
36 hrs
Tsg101-Flag: - - - - - + + +
Tal-HA: - - + + H695A - + H695A
Gag-GFP: GFP + - + + + + +
IB Ab to:
Virus
GFP
84
Virus
84
HA
Cell
84
HA
Cell
GFP
84

Fig. 7a

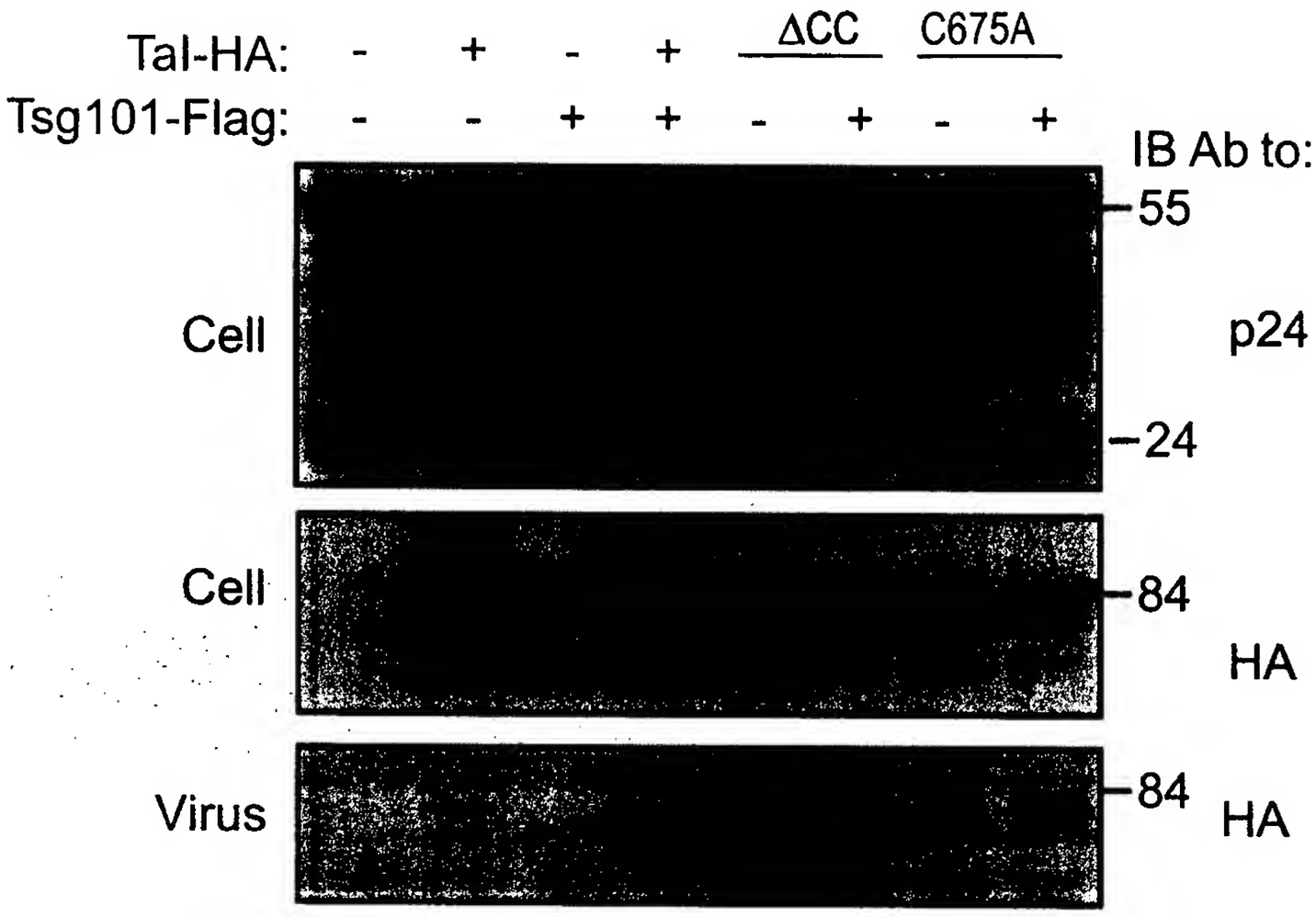

Tal-HA: - + - + ΔCC C675A
Tsg101-Flag: - - + + - + - +
IB Ab to:
Cell
55
p24
24
Cell
84
HA
Virus
84
HA

Fig. 7b

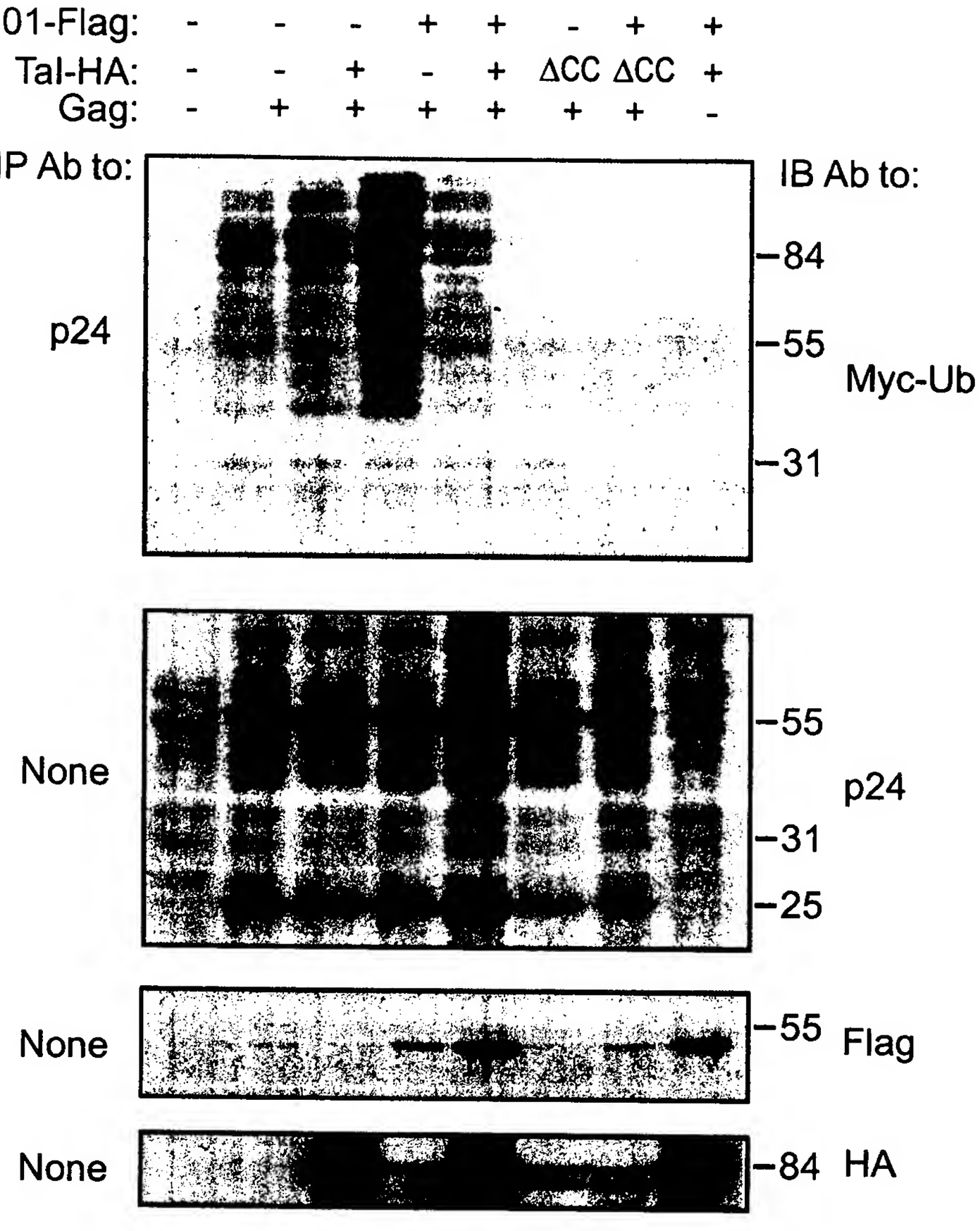

Tsg101-Flag: - - - + + - + +
Tal-HA: - - + - + ΔCC ΔCC +
Gag: - + + + + + + -
IP Ab to:
p24
IB Ab to:
84
55
Myc-Ub
31
None
55
p24
31
25
None
55
Flag
None
84
HA

Fig. 7c

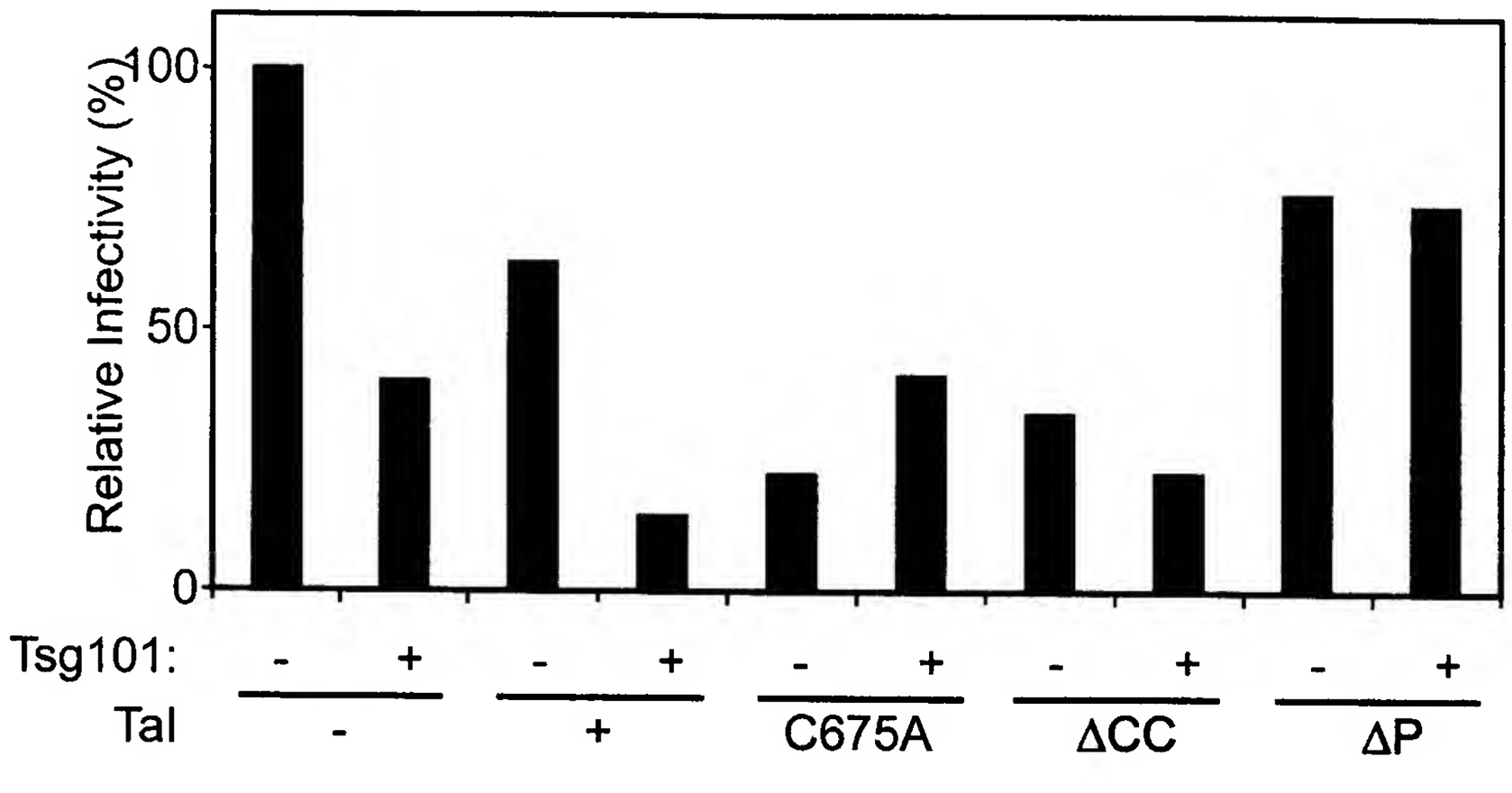

Relative Infectivity (%)
100
50
0
Tsg101:
-
+
-
+
-
+
-
+
-
+
Tal
-
+
C675A
ΔCC
ΔP

Fig. 7d

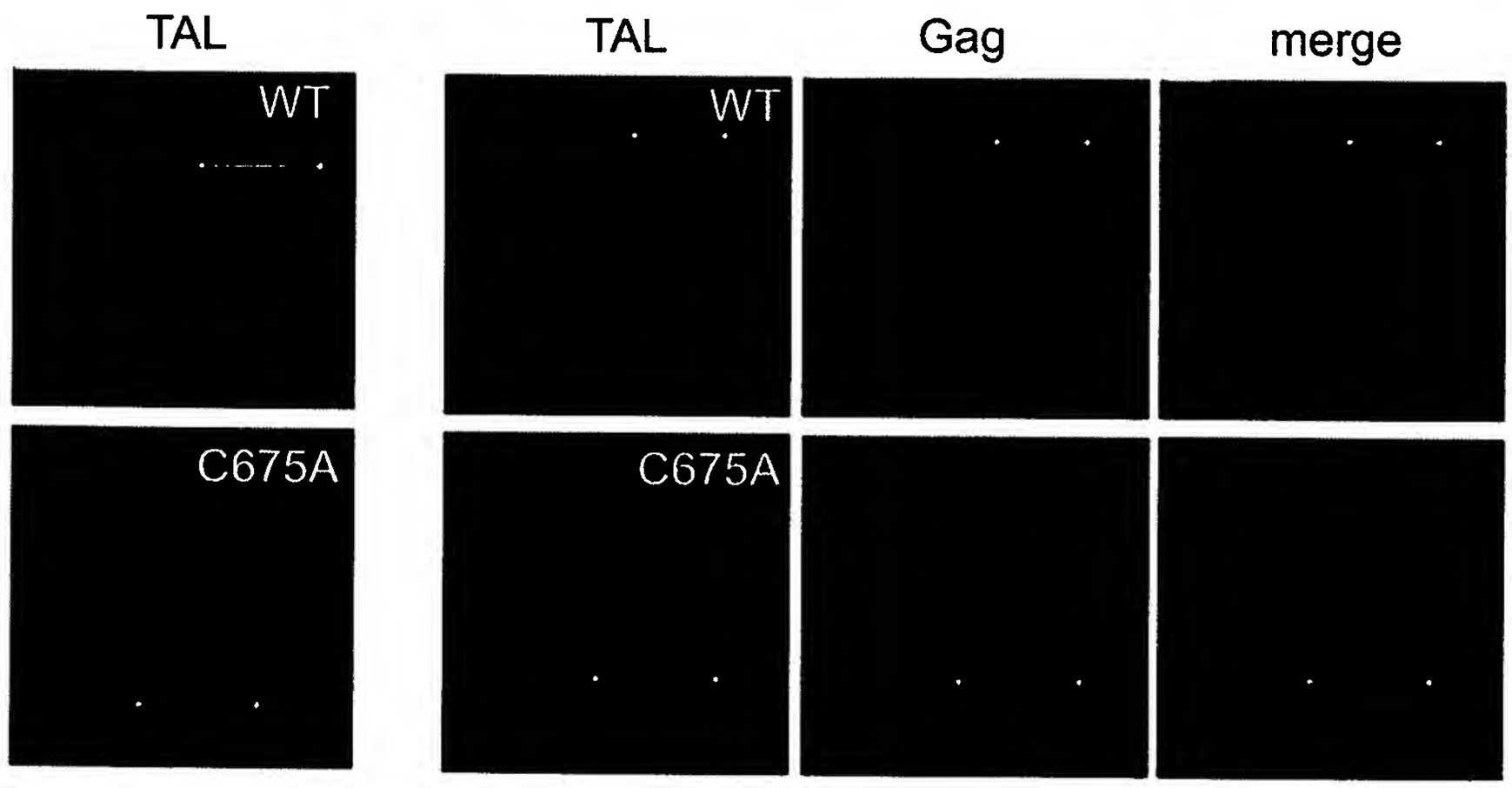

TAL
TAL
Gag
merge
WT
WT
C675A
C675A

Fig. 7e

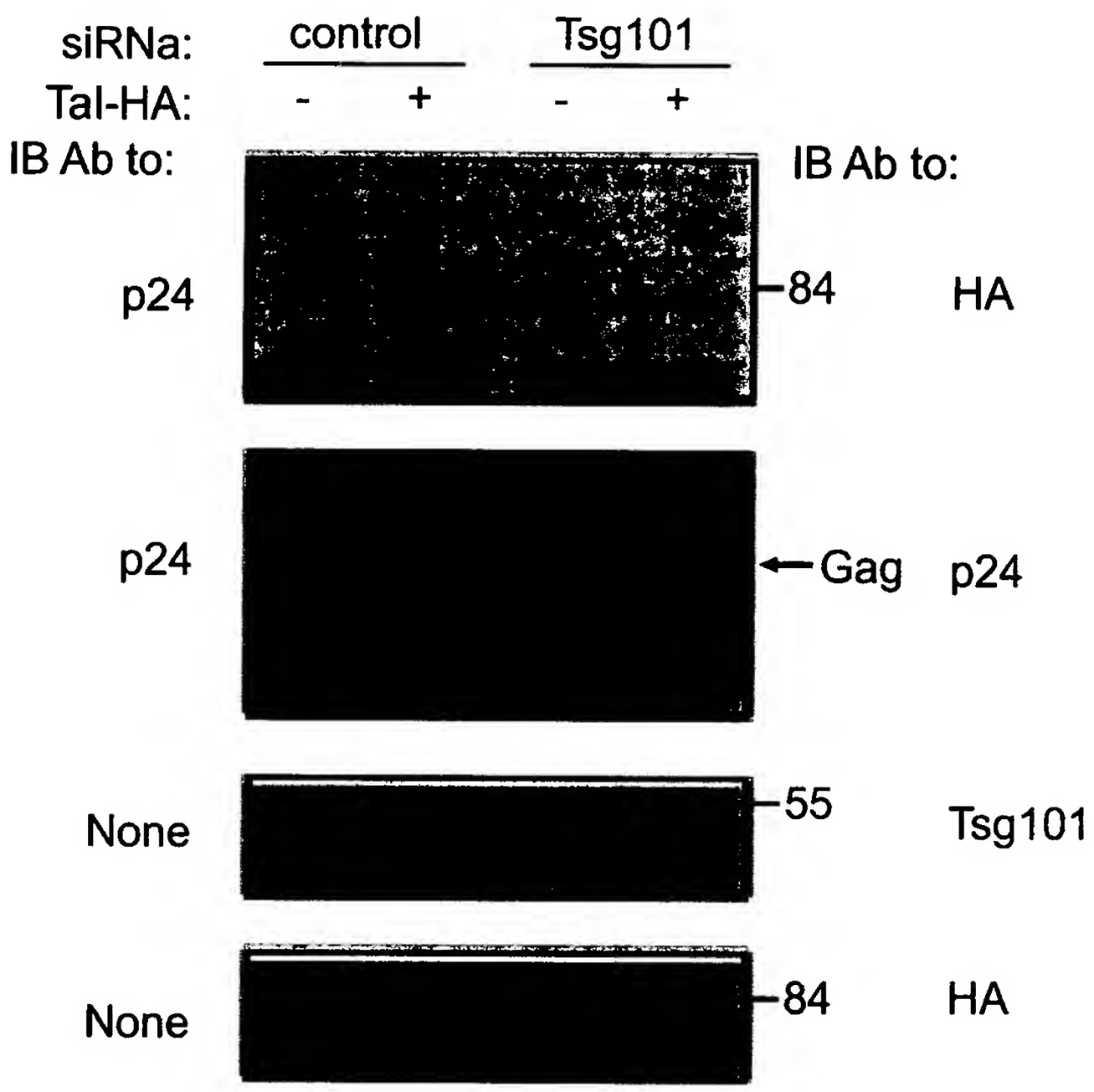

siRNa:
control
Tsg101
Tal-HA:
-
+
-
+
IB Ab to:
IB Ab to:
p24
84
HA
p24
Gag
p24
None
55
Tsg101
None
84
HA

Fig. 7f

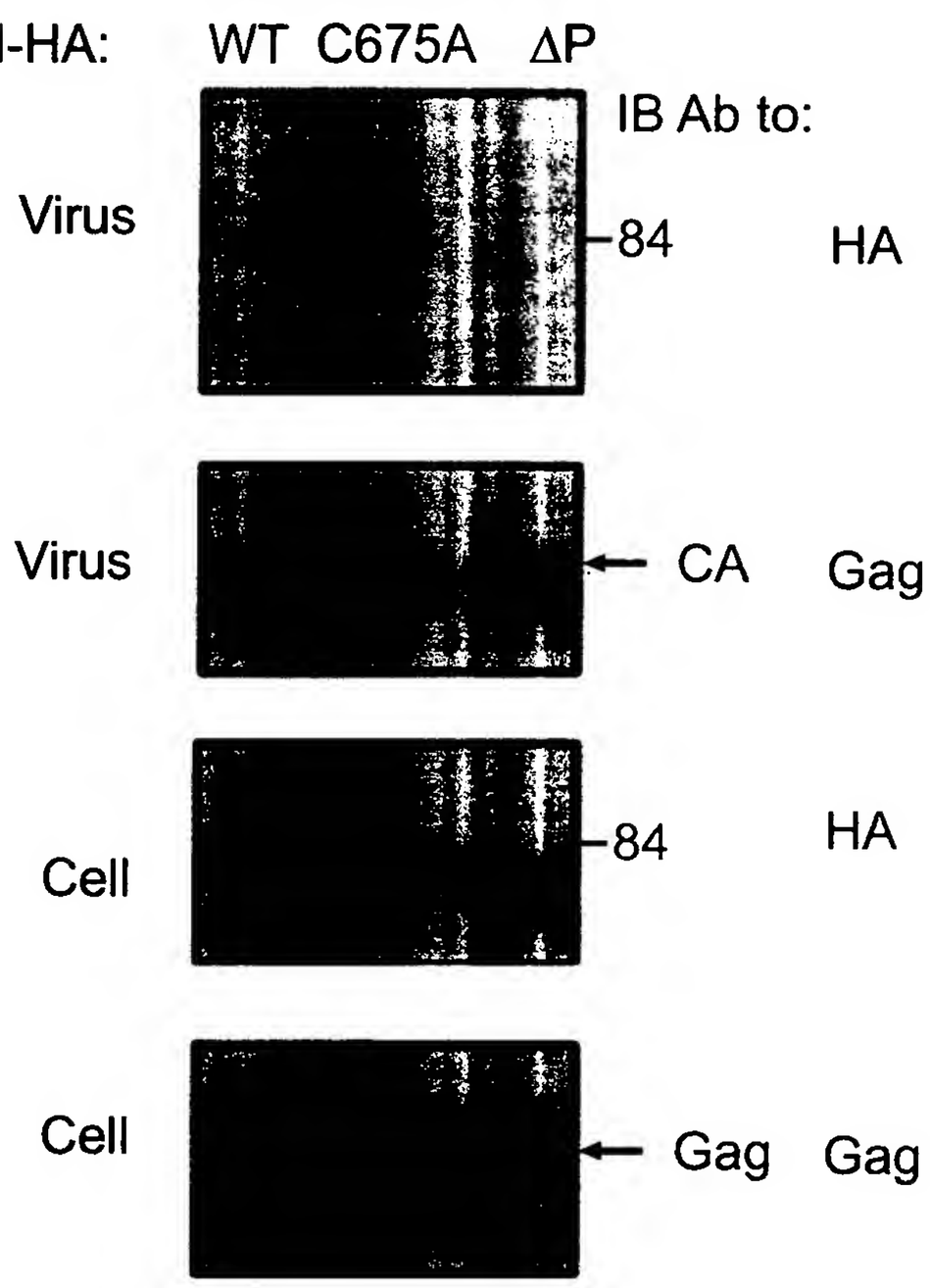

Tal-HA:
WT
C675A
ΔP
IB Ab to:
Virus
84
HA
Virus
CA
Gag
Cell
84
HA
Cell
Gag
Gag

Fig. 7g

Fig. 7h

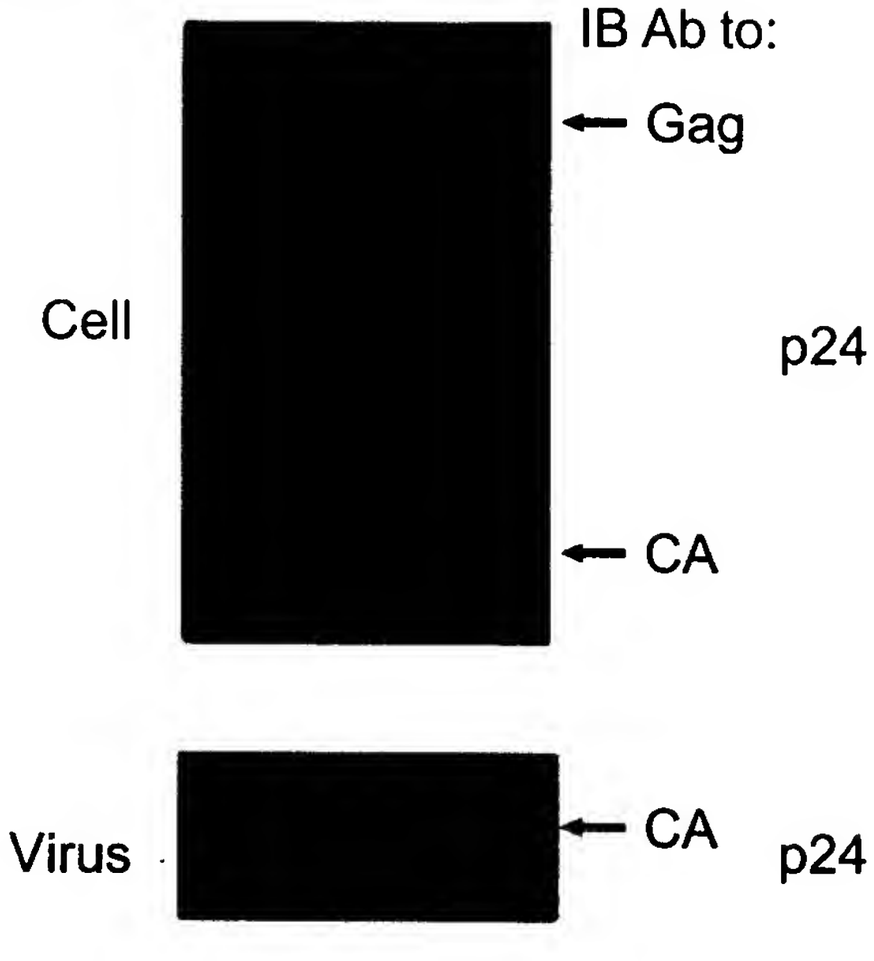
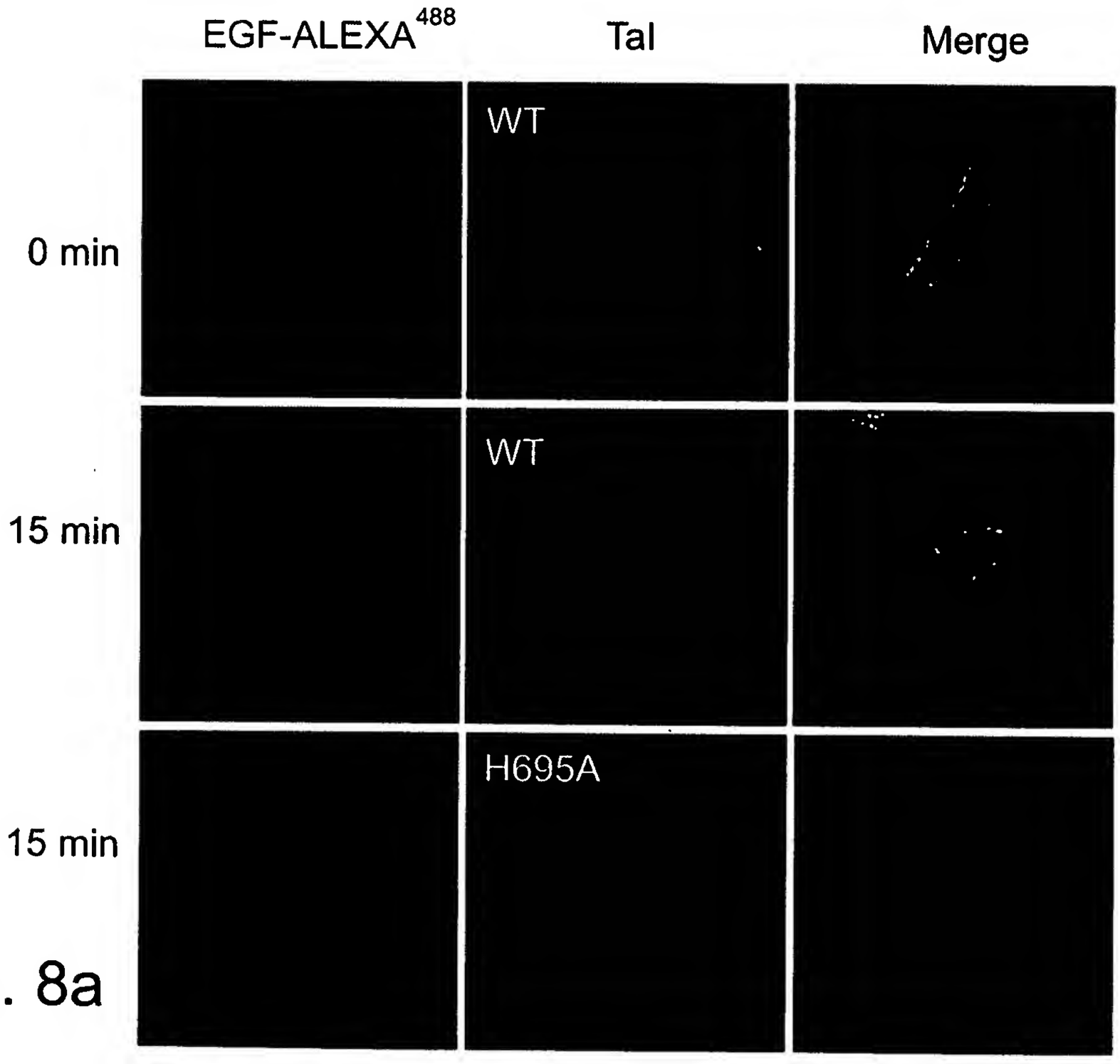

siRNA: Con.
Tal
IB Ab to:
Gag
Cell
p24
CA
Virus
CA
p24

Fig. 8a


EGF-ALEXA488
Tal
Merge
WT
0 min
WT
15 min
H695A
15 min

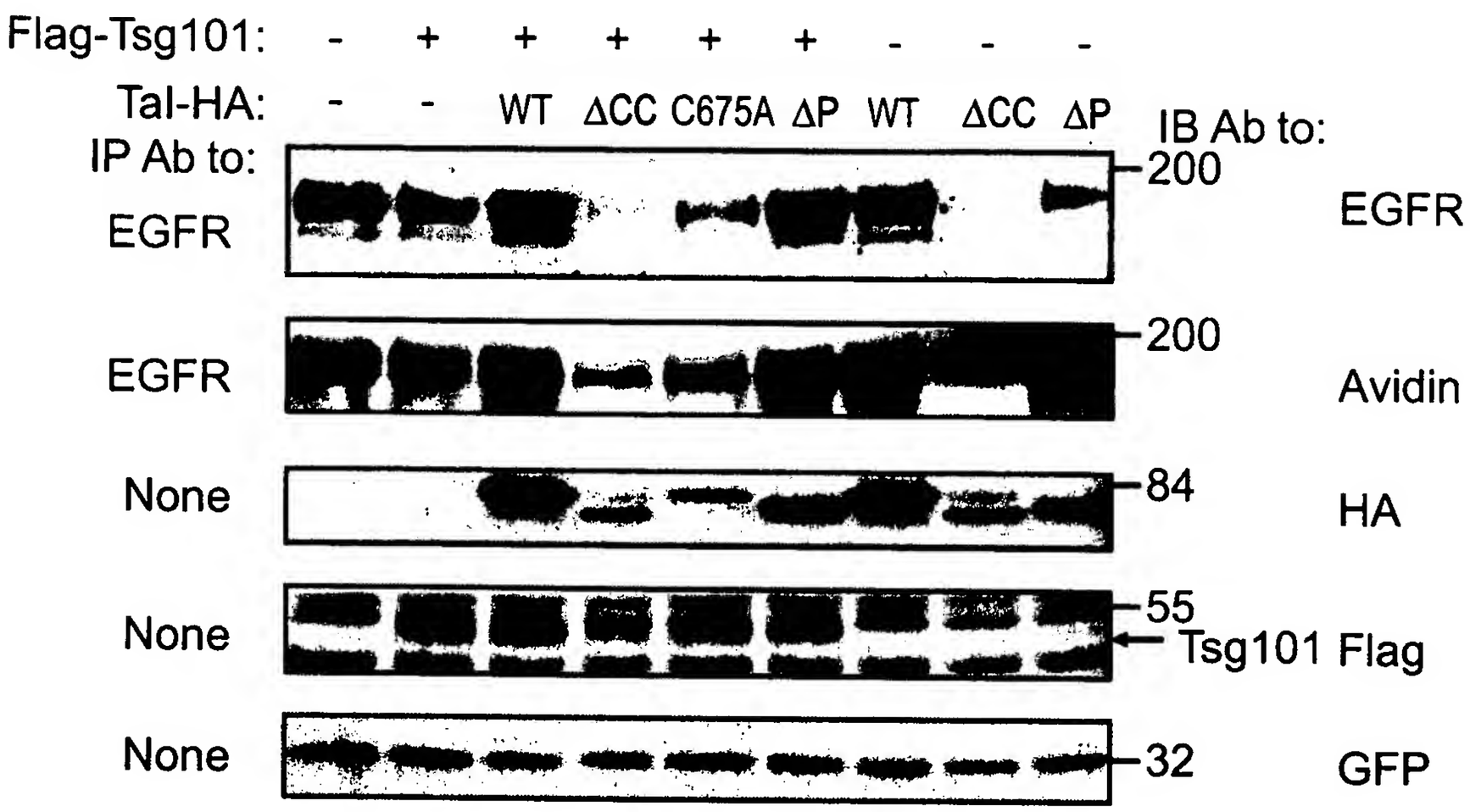

Flag-Tsg101: - + + + + + - - -
Tal-HA: - - WT ΔCC C675A ΔP WT ΔCC ΔP
IP Ab to:
IB Ab to:
EGFR
200
EGFR
EGFR
200
Avidin
None
84
HA
None
55
Tsg101 Flag
None
32
GFP

Fig. 8b

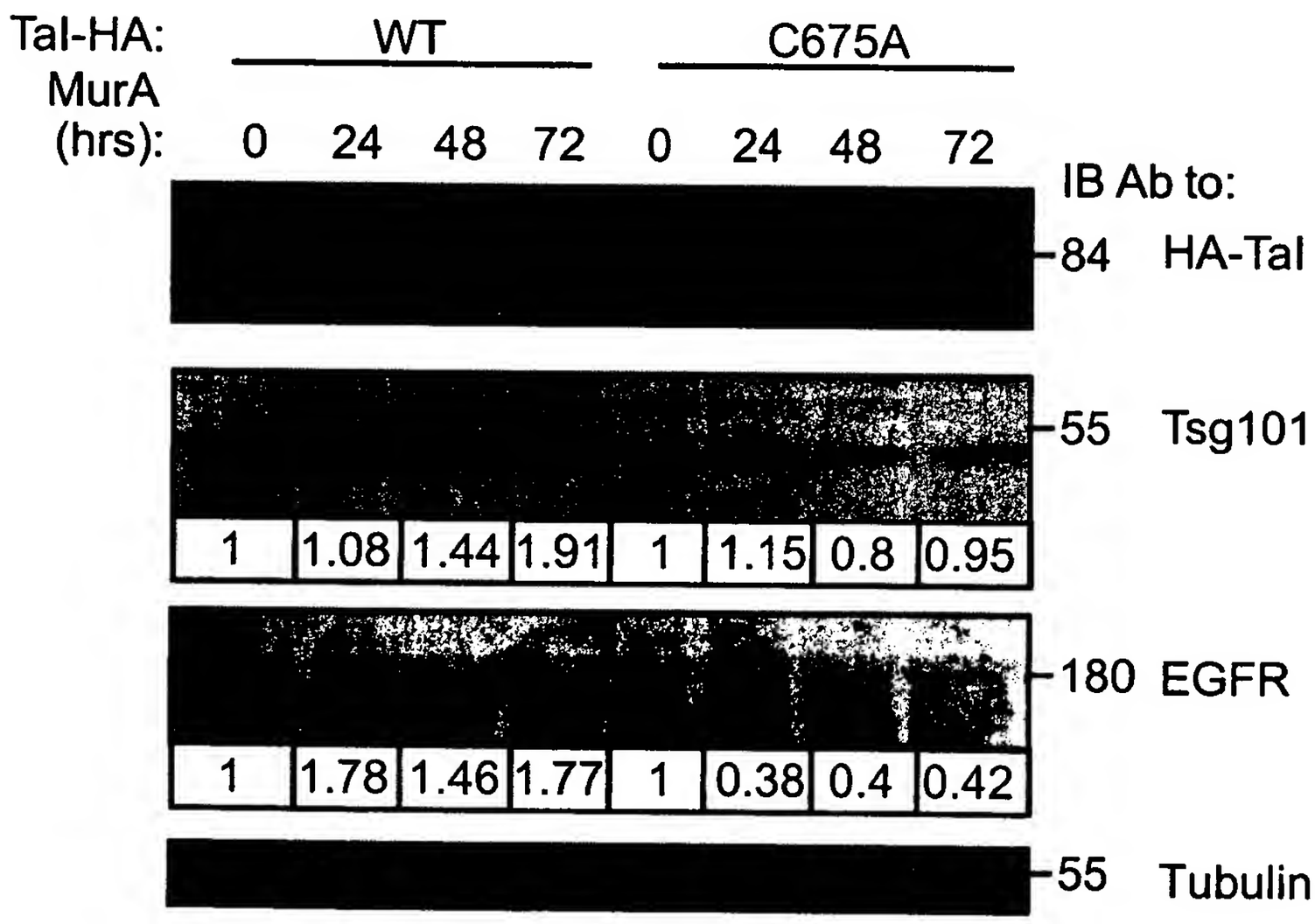

Tal-HA:
WT
C675A
MurA
(hrs):
0 24 48 72 0 24 48 72
IB Ab to:
84 HA-Tal
55 Tsg101
1 1.08 1.44 1.91 1 1.15 0.8 0.95
180 EGFR
1 1.78 1.46 1.77 1 0.38 0.4 0.42
55 Tubulin

Fig. 8c

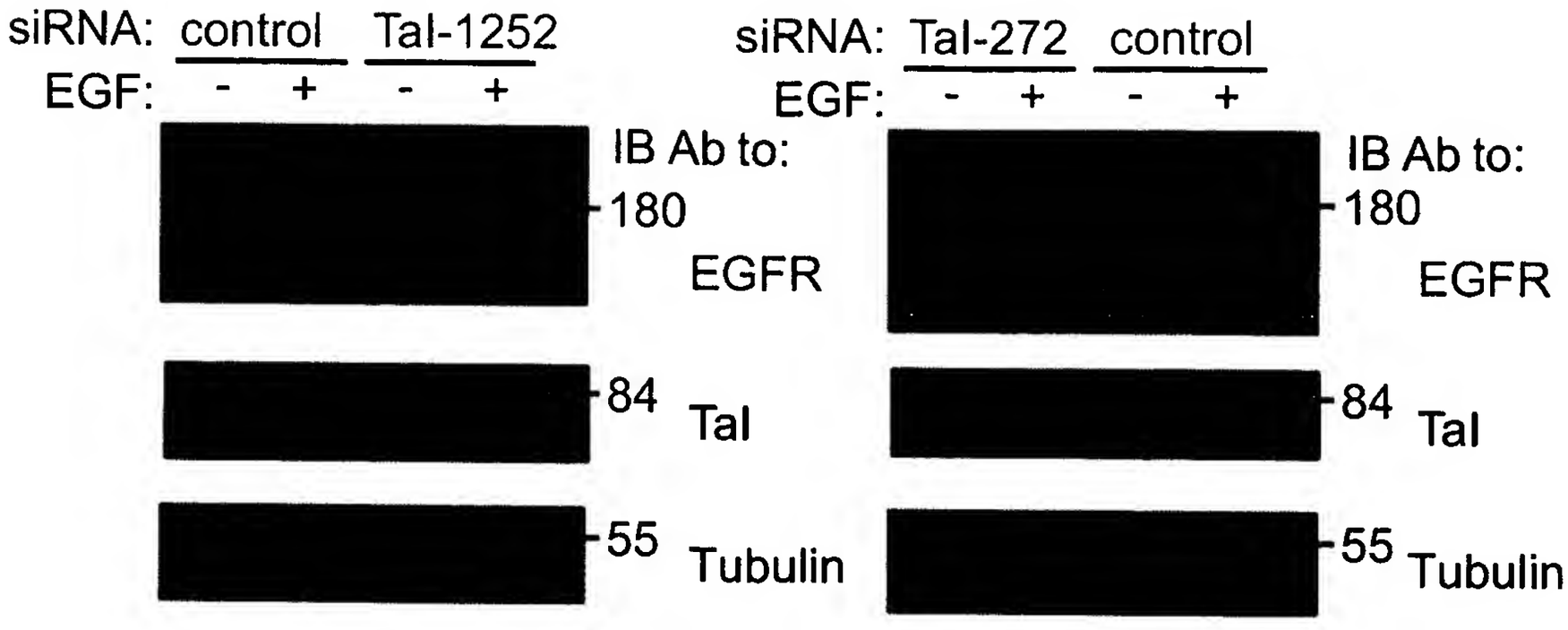

siRNA: control Tal-1252
EGF: - + - +
IB Ab to:
180
EGFR
84
Tal
55
Tubulin
siRNA: Tal-272 control
EGF: - + - +
IB Ab to:
180
EGFR
84
Tal
55
Tubulin

Fig. 8d

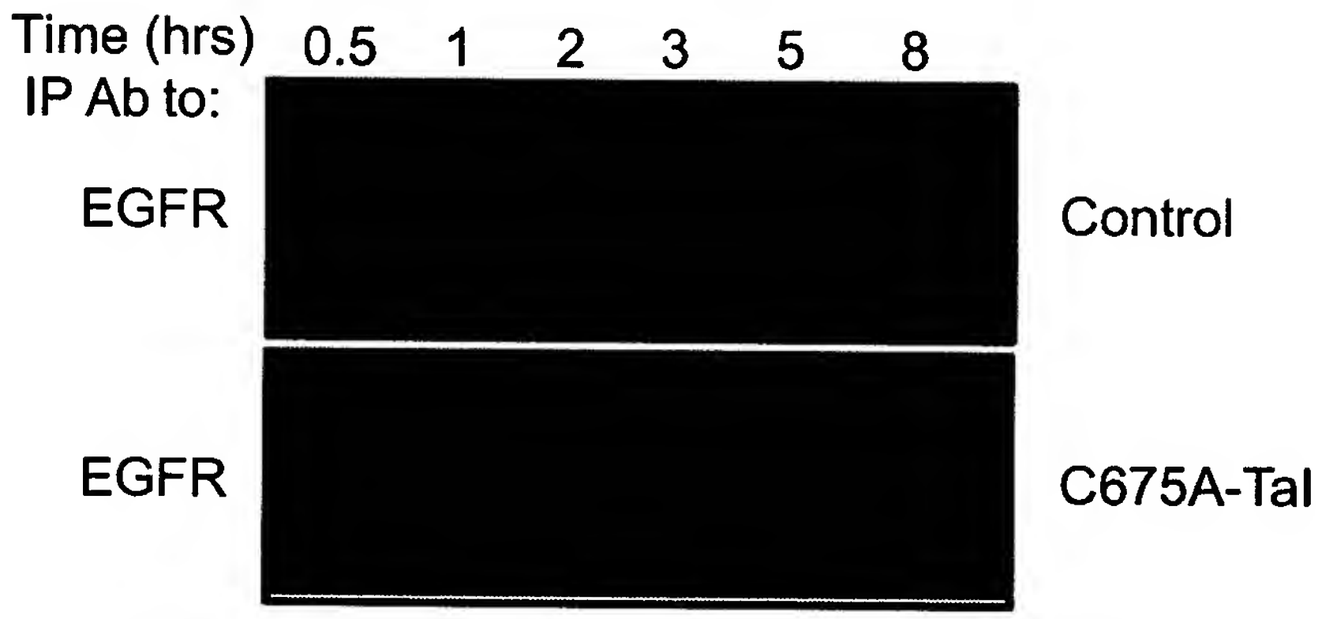
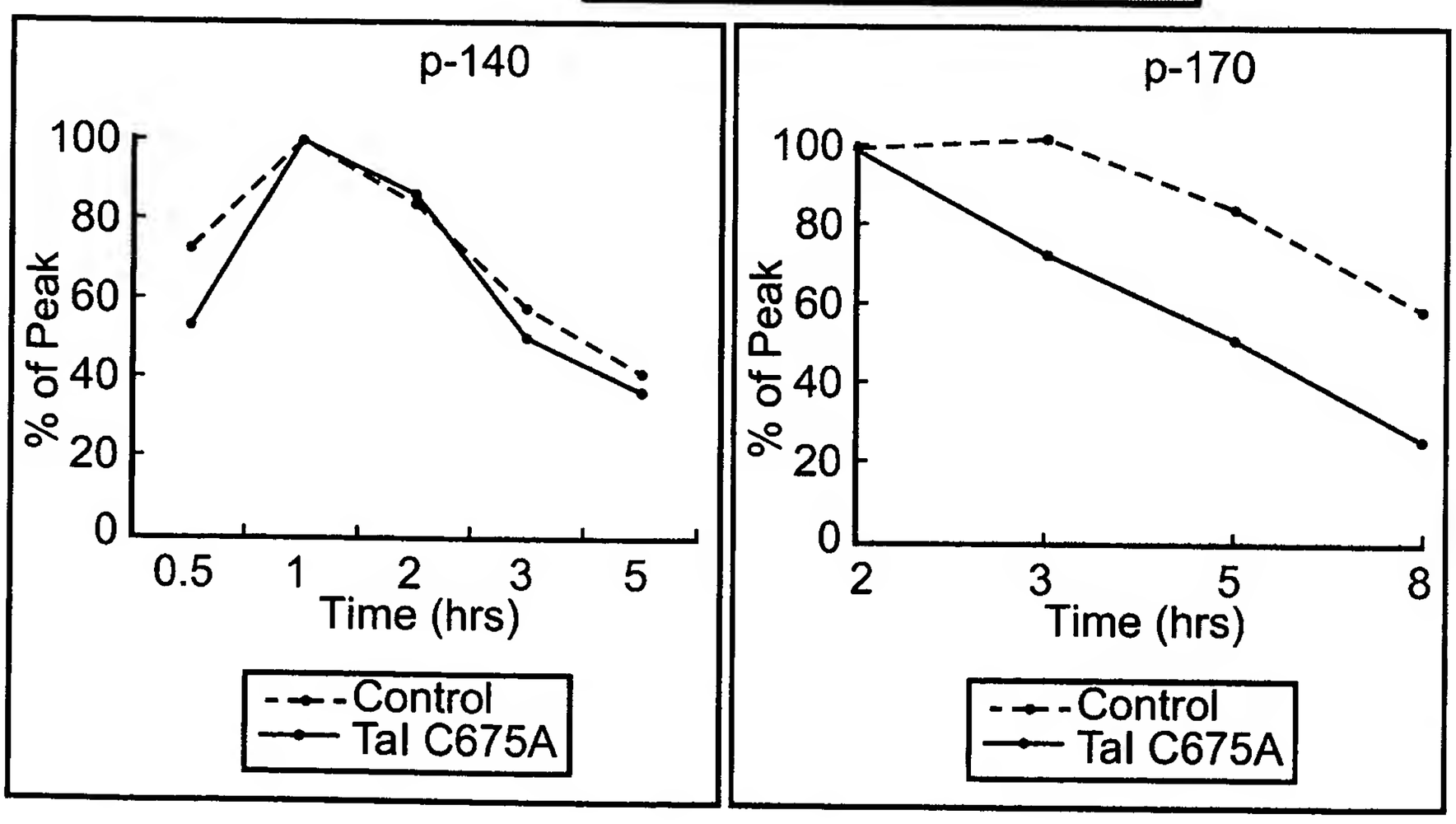

Time (hrs) 0.5 1 2 3 5 8
IP Ab to:
EGFR
Control
EGFR
C675A-Tal

Fig. 8e


p-140
100
80
60
40
20
0
% of Peak
0.5 1 2 3 5
Time (hrs)
Control
Tal C675A
p-170
100
80
60
40
20
0
% of Peak
2 3 5 8
Time (hrs)
Control
Tal C675A

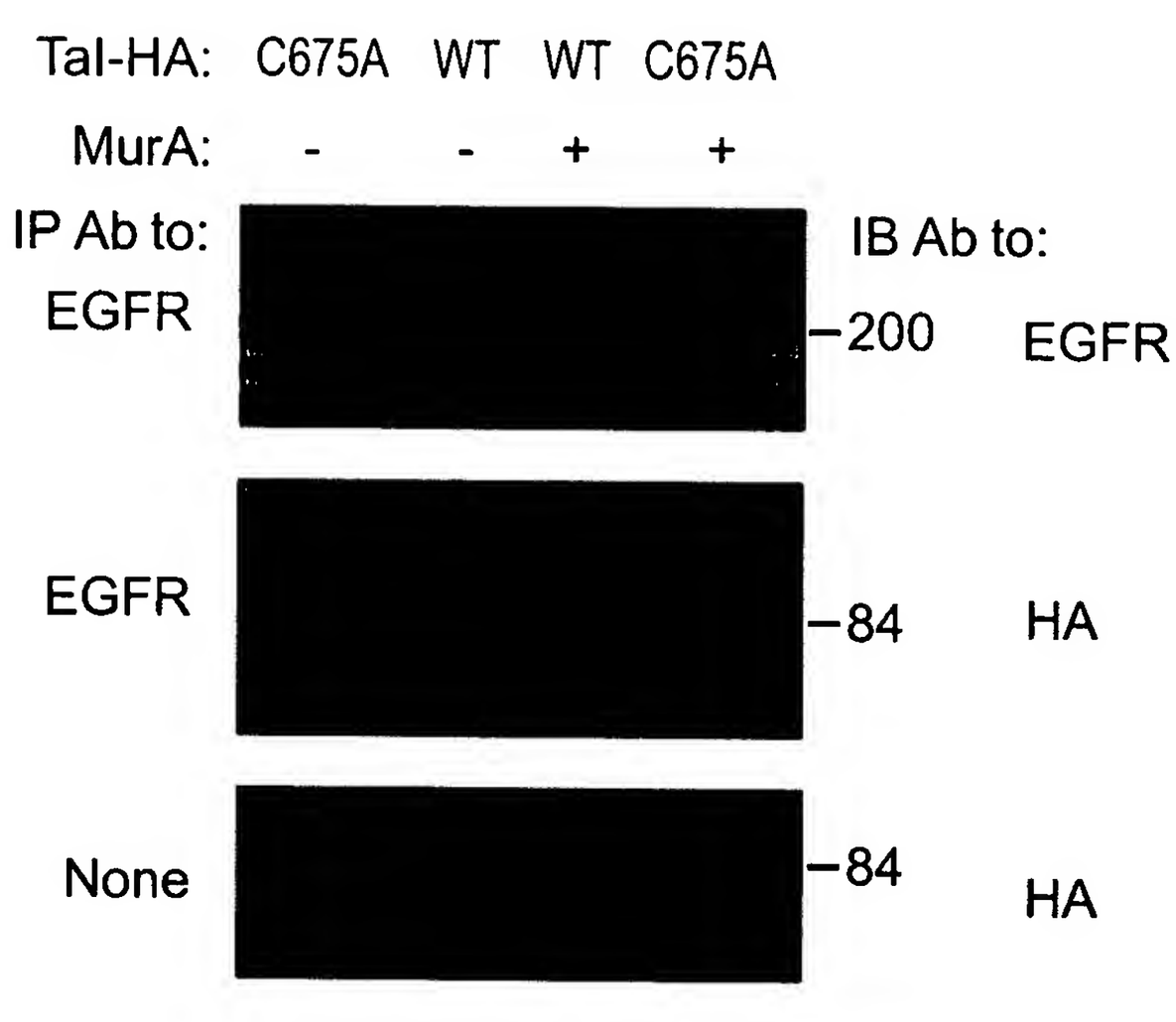

Tal-HA: C675A WT WT C675A
MurA: - - + +
IP Ab to:
EGFR
IB Ab to:
EGFR
200
EGFR
EGFR
84
HA
None
84
HA

Fig. 8f

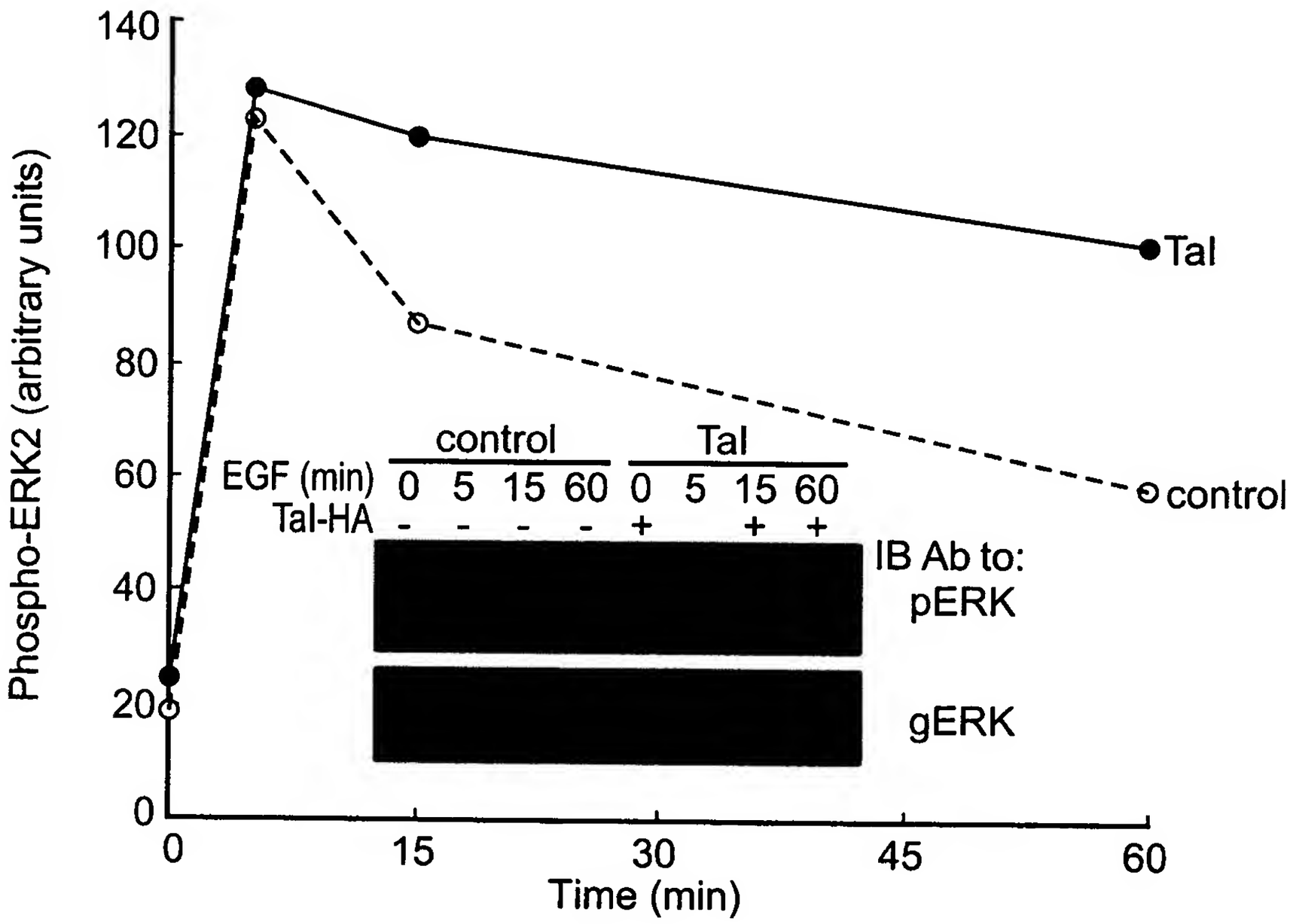

Phospho-ERK2 (arbitrary units)
140
120
100
80
60
40
20
0
0
15
30
45
60
Time (min)
Tal
control
control
Tal
EGF (min) 0 5 15 60 0 5 15 60
Tal-HA - - - - + + +
IB Ab to:
pERK
gERK

Fig. 8g

Fig. 9a

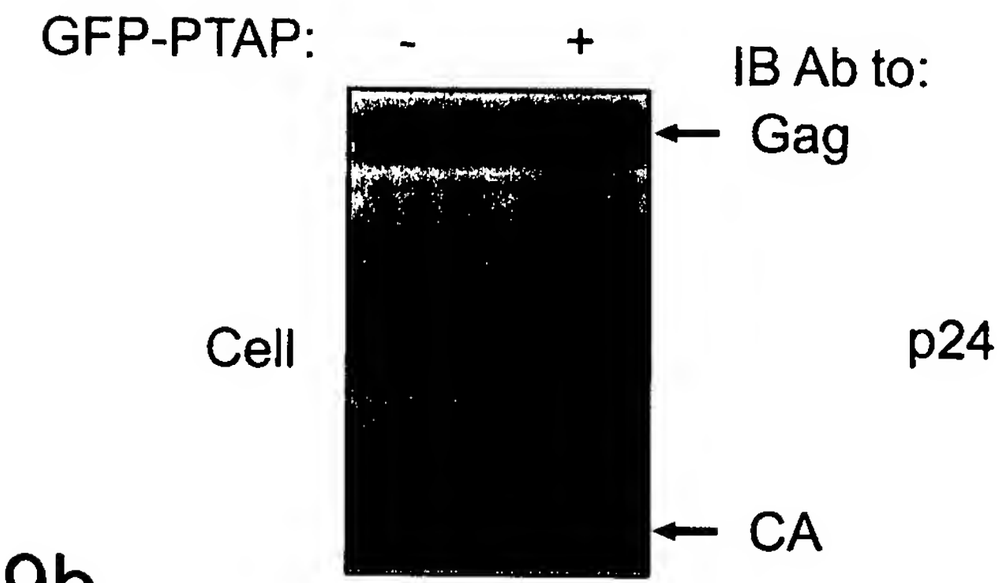


Fig. 9b

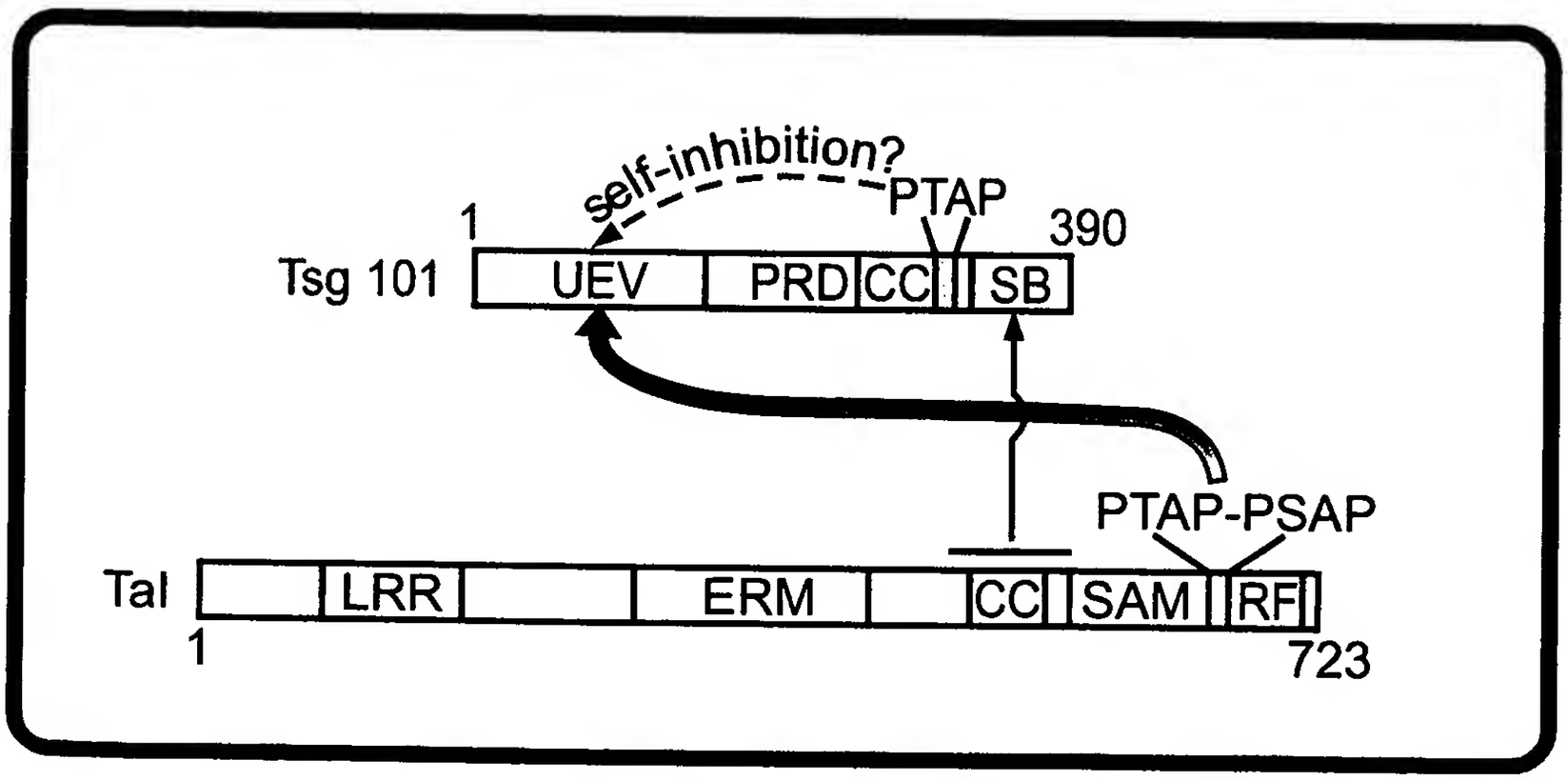


Fig. 10

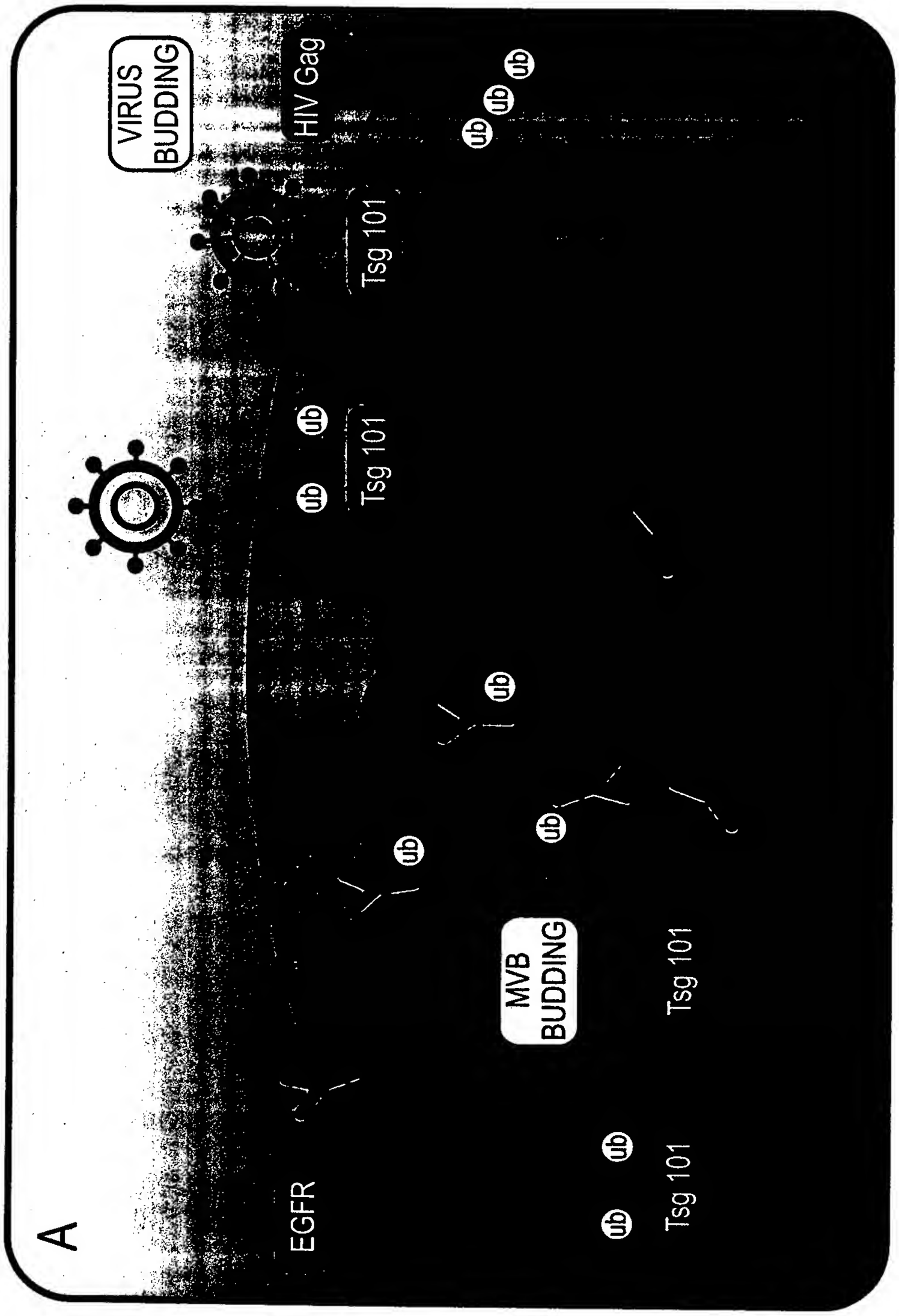

A
EGFR
ub
ub
Tsg 101
MVB
BUDDING
ub
ub
Tsg 101
ub
ub
Tsg 101
VIRUS
BUDDING
HIV Gag
ub
ub
ub
Tsg 101

Fig. 11